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Effect of Delayed Icing on the Quality Changes in Brackish water Shrimp *Penaeus monodon* During Ice Storage

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Abstract: The effect of delayed icing on the quality of ice-stored Brackish water shrimp (*Penaeus monodon*) was investigated by determining organoleptic, biochemical and bacteriological aspects. The live shrimp samples stored in ice immediately after harvest were organoleptically acceptable for 10 days while delay in icing for 4, 8 and 12 hrs shortened the shelf life to 7, 6 and 5 days, respectively. The initial pH of the live shrimp muscles was 6.63 which increased to 7.28 after 10 days of ice storage while in samples delayed in icing for 4, 8 and 12 h pH increased to 7.85, 7.93 and 7.95, respectively at the end of 10 days of ice storage. TVB-N value was increased from 5.88 to 32.76 mg/100g after 10 days of ice storage. The peroxide values in all the samples were lower than 8 meq/kg of oil upto 5 days of ice storage and then increased gradually with the lapse of storage period. The myofibrillar Ca^{2+}-ATPase activity in presence of 0.5M KCl showed the maximum remaining activity of 0.52 μmol pi/min.mg in live samples stored in ice immediately after catch. The myofibrillar solubility of samples immediately after catch was 80%, which decreased around 50% during 10 days of storage. On the other hand, the solubility of the samples kept at room temperature for different periods prior to icing were around 70% which decreased considerably to about 40% during 10 days of storage. The aerobic plate count (APC) increased considerably in the samples kept at ambient temperature for longer period prior to icing. The composition of bacteria in samples was Coryneforms (8.33%), *Bacillus* (7.40%), *Micrococcus* (16.66%), *Achromobacter* (8.33%), *Flavobacterium/Cytophaga* (25%), *Pseudomonus* (25%) and *Vibrio* (8.33%). Shrimp samples iced at 4,8 and 12 hrs delay dominated mostly by *Micrococcus* with 60%, 57.14% and 46.66%, respectively. During subsequent storage for 7 days, *Micrococcus* and *Achromobacter* were dominant in all the samples. However, *Enterobacteriaceae* was found in samples delayed 8 and 12 hr prior to icing.

Key words: Shrimp, delayed icing, quality loss

Introduction

Tiger shrimp (*Penaeus monodon*) is the most valuable sea food in international trade and considered to be the most important product of aquaculture in Bangladesh. Shrimp culture has been practiced in the eastern and southern coastal belt of Bangladesh. Traditionally the shrimp is harvested at night during high tide using traps or cast net and they are often not iced for 6-8 hours until transported to the collection center. Ice is used only when a bulk quantity is gathered and finally transported to the processing plants. The collection of raw material passes through a number of channels: primary, secondary and final delivery to the industry. Serious quality deterioration has been reported at different stages of handling and transportation and major causes of rejection are black spot, broken, soft shell and peeled/rotten, discolour, moulted shrimp, filth, dehydration, foreign material and kerosine. The problems associated with the quality loss are probably lack of landing facility, dumping in dirty earthen floor, inadequate drainage system, poor water quality, inadequate washing system, inadequate insulated storage facility, lack of quality consciousness and no insulated refrigerated transport. Among the above mentioned factors which influenced the quality loss, the most important factor is probably the exposure of harvested shrimp to higher ambient temperature for a longer period that drastically reduce the shelf life of shrimp. It is clear that there is not much control on raw material supplies with respect to maintaining cold chain from the harvesting point to the processing plants. In most cases sufficient care is not taken by the supplier in between the time of entry and delivery of raw material to the plants receiving counter which offers possibilities for further deterioration in quality. The shrimp processing industry in Bangladesh is facing with the lack of adequate supply of raw material. There is a competition among traders for collection of raw material which is so high that the shrimp of any quality is sold for processing in the industry. The available information suggest that the effect of delayed post-harvest handling exerts serious threat on quality of fish and shellfish (Barlie *et al*., 1985; Reilly *et al*., 1985 and Dawood *et al*., 1986). In the previous study, changing in fresh water Prawn *Macrobrachium rosenbergii* and *Penaeus monodon* were investigated under various storage conditions. Both species were found in organoleptically acceptable conditions for 6 to 7 days and the delayed icing considerably shortened the shelf life of the fresh water Prawn *Macrobrachium rosenbergii* (Kamal *et al*., 2000; Rahman, *et al*., 2000; Rahman *et al*., 2001a, 2001b). However, very little is known on the quality of *P. monodon* of this tropical region where raw materials are collected for processing after various stages of handling and transportation and without maintaining proper cold chain. There is no precise information on the volume of post-harvest quality losses because of lack of detailed research /surveys. Widespread ignorance of the factors affecting quality and no effective measure to overcome the problems leads to significant economic loss. With the views mentioned above, the present study has been undertaken to evaluate the effect of delayed icing on quality loss of *P. mondon* by determining the organoleptic, biochemical and bacteriological aspects.

Materials and Methods

Materials: Brackish water giant shrimp *Penaeus monodon* (average size 30-35/kg) were collected alive from one commercial farm of Paikgacha, under Khulna district. The samples were divided into four groups. One group of live samples were ice-stored immediately after harvest and remaining sample groups were ice-stored after 4, 8 and 12 hrs

of harvest and packed separately in polythene bags. The samples were transported to the Laboratory of the Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh in an insulated box using ice shrimp in the ratio of 1:1. At selected time interval, and desired number of samples were used to assess the effect of delayed icing on degree of freshness by evaluating organoleptic, biochemical and bacteriological aspects.

Organoleptic assessment: The organoleptic method used in this study is based on the existing procedure of the Fish Inspection and Quality Control Service (FIQC) of the Department of Fisheries (DOF), the Government of Bangladesh. A member panel was constituted to evaluate the organoleptic quality changes of giant tiger shrimp (*Penaeus monodon*) on the basis of odour, texture, colour of shell, colour of flesh and general appearance of shrimp. The quality was evaluated by grading the shrimp using the score from 5 to 25 . The grade defined in terms of the total number of points were: 22 to 25 considered as very good or excellent; 19-21 good, 14-18 acceptable; 8-13 bad and 5 to 7 very bad condition.

Total volatile base nitrogen (TVB-N) determination: Total volatile basic nitrogen (TVB-N) of the samples was determined according to the method described by the Official Journal of the European Communities (EC, 1995).

pH measurement: Two grams of peeled shrimp was homogenized with 10 ml distilled water in a blender and the pH was measured using a pH meter (Corning Model 250).

Peroxide value: The peroxide value was determined according to the method of Limados Santos *et al.* (1981).

Assay of specific ATPase activity: The reaction mixture for the Ca^{2+}- ATPase assay contained 25mM Tris, 5mM $CaCl_2$, 0.5M KCl and 0.25 mg myofibril per ml. The ATPase activity was measured at 25°C for 6 min. After preparation of the reaction mixture, an appropriate quantity of myofibril suspension was pipetted to the reaction mixture followed by 2 min. pre-incubation. The reaction was started by the addition of 1mM ATP and 2 ml portion of the reaction mixture was withdrawn at different time intervals. Adding 1ml of 15% trichloroacetic acid stopped the reaction. The supernatant obtained by 5min centrifugation at 3000 X g was analyzed for the liberation of inorganic phosphate (Pi) by a method described by Fiske and Subba Row (1925).

Solubility of myofibrillar proteins: Myofibrillar proteins were extracted from isolated myofibrils with 0.6M KCl-0.03 M Tris-HCl at pH 7.5. The suspension was stirred gently and kept over night at 4 °C. Then the solution was centrifuged at 900x g for 30 min and protein content in the supernatant was determined by the biuret method (Gornall *et al.*, 1949).

Microbial studies:

Preparation of shrimp muscle: The shrimps were deheaded carefully and the shell and vein (intestine) was separated aseptically from the muscle and then weighed and finally chopped by scissors on a sterile watch glass. The stock suspension of the muscle was prepared in physiological saline (0.85% NaCl) using a sterile warring blender and desired dilutions were made according to decimal dilution method. Strict aseptic procedures were followed in every step of the study.

Total aerobic plate count: Total aerobic plate count expressed as colony forming units per gram of shrimp muscle (cfu/g) of the representative samples were determined by standard plate count method on plate count agar (High-media, India) according to Collins and Lyne (1976).

Total coliform count: Plating the same samples used for total plate count in a previously prepared Violet Red Bile Agar (VRBA) plates was done for total coliform count. Bright pink coloured colonies developed on this selective agar medium was counted and used for the calculation of total coliform per gram of muscle. An alternate method of Most Probable Number (MPN) method (Collins and Lyne, 1976) was also used to confirm results of plating method.

Isolation and identification of bacteria: In order to isolate the bacterial strains for identification, colonies developed from diluted samples on agar plates were differentiated on the basis of their morphological characteristics and later typical representative colonies were picked up by means of sterile wire-loop for streaking on agar plates. The streaked agar plates were incubated at 30 °C for 2 days. Discrete colonies from the streaked agar plates were transferred to agar slants as a pure culture. After 2 days of incubation, smears were prepared on glass slides and stained by the Gram's method and examined microscopically for their purity.
Identification and generic classification of the bacterial isolates was done according to an outline of the sequence of tests in the screening of culture described by Shewan *et al.* (1960).

Results and Discussion

The changes in sensory properties of *Penaeus monodon* ice-stored after delaying icing for various time interval are given in Fig. 1. The freshness was judged by the panel of experts on the basis of organoleptic characteristics such as appearance, textural condition, colour and odour. The samples stored in ice immediately after catch were organoleptically acceptable for 10 days while delayed icing of 4, 8 and 12 hrs shortened the shelf life to 7, 6 and 5 days, respectively. The delayed icing resulted in increased melanosis in comparison to that of the samples iced immediately after catch. This is in agreement with that of Reilly *et al.* (1984), reported for *Penaeus monodon* where the storage life in ice reduced by approximately one day for every hour delay in icing .
Fig. 2 shows the effect of delayed icing on the changes in pH of the shrimp muscles during subsequent storage. The initial pH of the muscles was 6.63 which increased to 7.28 after 10 days of storage in samples stored in ice immediately while the pH increased to 7.85, 7.93 and 7.95 during storage in samples kept at high ambient temperature (31°C) for 4, 8, 12 hrs prior to icing. However, on the basis of sensory evaluation the pH value above 7.3 was found unacceptable for consumption or spoiled. The pH 7.35 is the upper limit of acceptance as described by Asian Food Handling News letter Oct. 1986. The pH of shrimp has been suggested as a good index of freshness (Cheng, 1977; Cheng and Lain, 1979). The pH value of 7.8 was reported to critical level which suggest that spoilage has commenced (Cheng and Lain, 1979).
Changes in TVB-N values during delayed icing and subsequent storage are shown in Fig. 3. The initial average TVB-N value was 5.88 mg/100g in samples stored in ice immediately after catch that increased to 32.76 mg/100g after 10 days which is slightly higher than the recommended value of 30 mg/100g as reported previously (Cobb *et al.*, 1973; Connell, 1995). As shown in Fig. 3, TVB-N formation in the samples kept at high

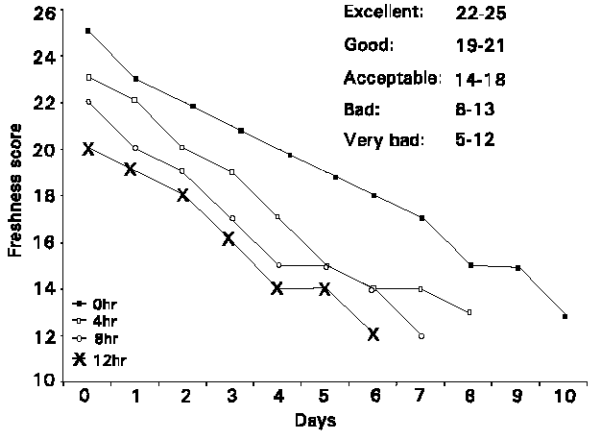

Fig. 1: Effect of delayed icing on the organoleptic quality of *Penaeus monodon* during subsequent ice storage.

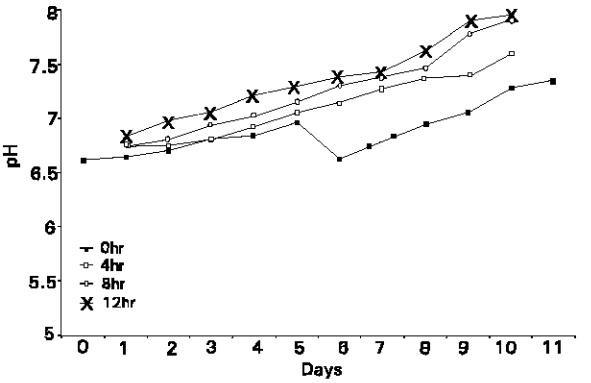

Fig. 2: Changes in pH of *P. monodon* during ice storage after delayed icing.

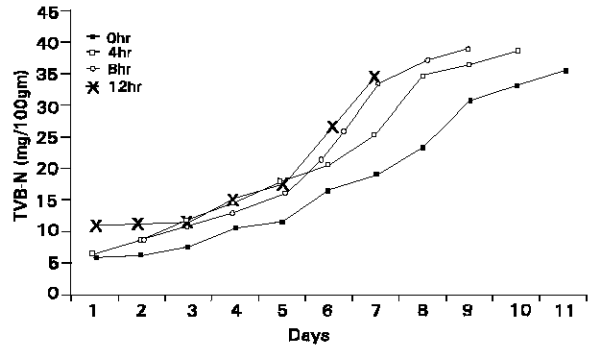

Fig. 3: Changes in TVB-N of *P. monodon* during ice storage after delayed icing.

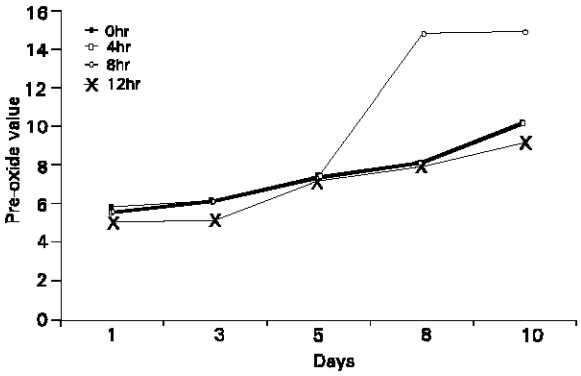

Fig. 4: Pre-oxide value of *P. monodon* during ice storage after delayed icing.

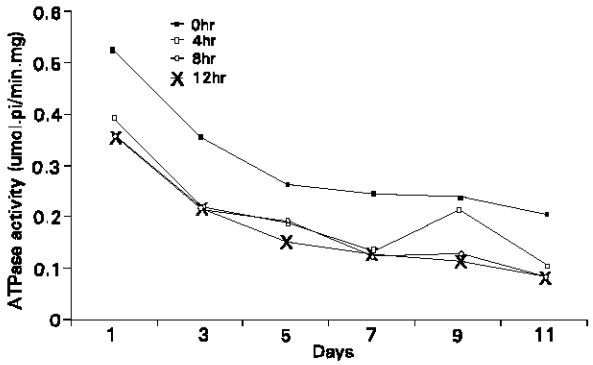

Fig. 5: Changes in myofibrillar ATPase activities in *P. monodon* during ice storage after delayed icing.

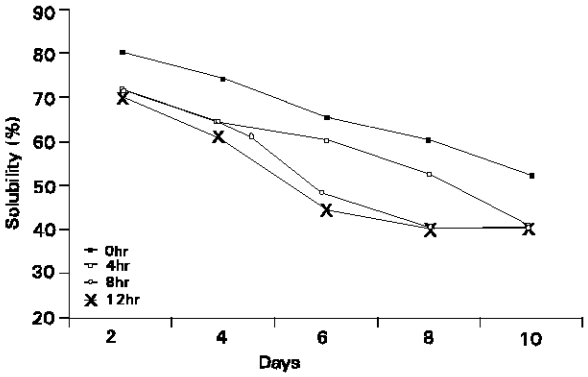

Fig. 6: Solubility of *P. monodon* during ice storage after delayed icing.

ambient temperature (31 °C) for 4, 8 and 12 h prior to icing were more advanced than the samples stored in ice immediately after catch where the TVB-N values exceeded the acceptance limit within 5-7 days. The result has indicated that the formation of TVB-N value is much rapid in samples exposed to high temperature for a longer period after catch. There is a correlation between TVB-N values and organoleptic scores where the TBV-N values in samples exceeded the recommended values were in organoleptically unacceptable conditions. However, the present study suggests that temperature control is very important to maintain the quality of shrimp.

The peroxide values in all the samples were lower than 8 meq/kg of oil up to 5 days of storage but the values increased gradually with the lapse of storage period (Fig. 5). The highest

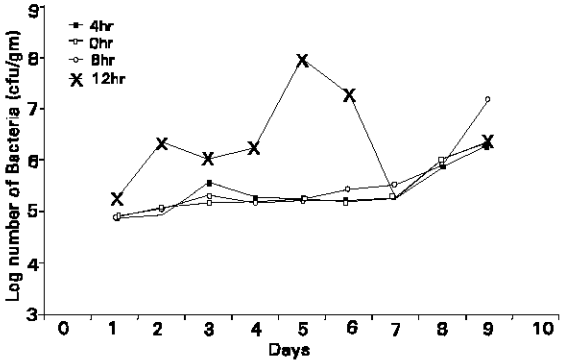

Fig. 7: Effect of delayed icing on the quantitative changes in Bacterial load of *Penaeus monodon* during ice storage.

values of 15-17 meq/kg of oil were obtained after 10 days of storage in samples delayed for 8 and 12 hrs prior to icing but the values were within the acceptable limit of 10-20 meq/kg of oil as suggested by Connell (1980). The peroxide values were comparatively low in samples stored in ice immediately after catch than those kept at room temperature for a longer period before icing. The lower peroxide value in all the samples even at the stage of rejection is related to low lipid content of the shrimp.

Studies were conducted on the changes in myofibrillar ATPase activity to assess the effects of delayed icing on muscle protein denaturation of Brackish water shrimp (Fig. 5). The myofibrillar Ca^{2+}-ATPase activity in presence of 0.5M KCl was measured in all the samples after one day of ice storage. The maximum remaining activity of 0.52 μmol pi/min.mg was obtained from samples stored in ice immediately after catch, while the activity in samples delayed for different time interval prior to icing were low. However, the ATPase activity declined significantly with the lapse of storage period in all the samples under various storage conditions but the activity of the ice stored samples immediately after catch declined slowly. Since Ca^{2+}-ATPase activity is a good indicator of the integrity of the myosin molecule, the decreased activity of the samples may be originated from the changes in myosin structure (Yasui *et al.*, 1958). It is generally accepted that high ambient temperature accelerates the autolytic process and bacterial action rather than the effect of temperature alone. Some lysomal proteases might be responsible for degradation of certain muscle proteins thus decreasing myofibrillar ATPase activity in such storage conditions (Dutson, 1983; Ouali *et al.*, 1987).

The changes in soluble proteins in shrimps ice-stored with delay in icing are shown in Fig. 6. The myofibrillar solubility of 2 days ice stored shrimp proteins immediately after catch was 80% which decreased to around 50% during 10 days of storage. On the other hand, the solubility of the samples kept at room temperature for different periods prior to icing were around 70% which decreased considerably to about 40% during 10 days of storage. But the decrement of solubility was rapid with the lapse of storage period in samples those kept longer for period at room temperature prior to icing. The results obtained from this study revealed that denaturation of myofibrillar proteins takes place during storage period.

Fig. 7 shows the effect of delayed icing on the bacterial load (APC) of shrimp sample. The APC count increased considerably with the samples kept at ambient temperature for longer period prior to icing. At 0 hour of harvest APC of the shrimps sample was 7.6×10^4cfu/gm, which increased to 1.90×10^6cfu /gm over the period of 9 days in ice storage . On the

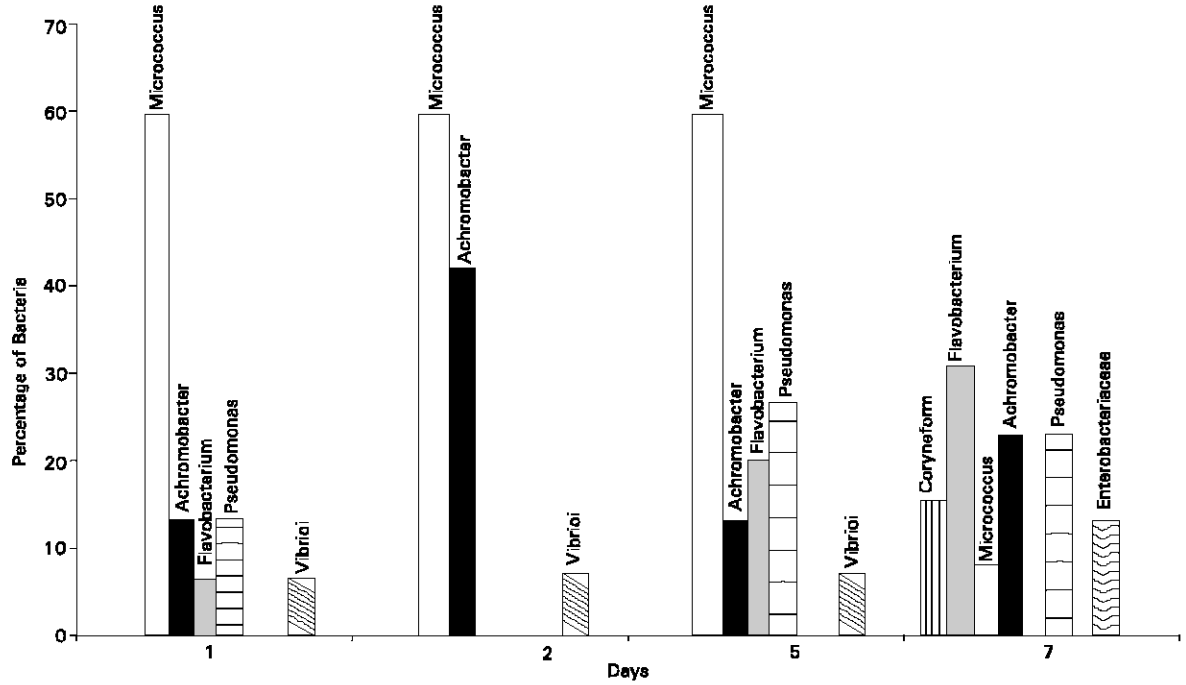

Fig. 8: Effect of delayed icing (4 hour delay) on the generic distribution (%) of bacteria in *P. monodon* at different duration of storage.

other hand, the initial APC of shrimp iced after 4, 8 and 12 hrs after harvest had $8.32x10^4$, $8.71x10^4$ and $1.78x10^5$ cfu/gm. After 9 days of storage, APC count in samples delayed for 4 and 8h prior to icing increased to 2.29×10^6 and 1.6×10^7 cfu/gm, respectively, while in samples delayed by 12h, the APC count increased to 1.03×10^8 in 5 days although the count decreased to some extent with further storage period. Fig. 8 shows the effect of delayed icing on the generic distribution (%) of bacteria in *P. monodon* during ice storage. The composition of bacteria in samples preserved in ice immediately after harvest were Coryneforms (8.33%), *Bacillus* (7.40%), *Micrococcus* (16.66%), *Achromobacter* (8.33%), *Flavobacterium/Cytophaga* (25%), *Pseudomonus* (25%) and *Vibrio* (8.33%). Shrimp samples iced 4,8 and 12 hrs delay dominated mostly by *Micrococcus* 60%, 57.14% and 46.66%, respectively. During subsequent storage for 7 days, *Micrococcus* and *Achromobacter* were dominant in all the samples. However, *Enterobacteriaceae* was found in samples delayed 8 and 12 hr prior to icing. There were some microflora that could not be identified in the distribution list. Previous report (Campbell and Williams, 1952) showed a shift of more than half of the original bacterial species made up of *Bacillus, Micrococcus and Flavobacterium* to *Achromobacter* and *Pseudomonas* (total 98%) after 16 days of ice storage of Peneaus. sp whereas Cook (1970) showed no consistent changes in bacterial contents during the initial decrease on headless brown shrimp (*P. aztecus*), but an increase of *Pseudomonas* species (80-100%) with increased time on ice. The organoleptic property shows that the delayed icing has shortened the shelf life of *P. monodon*.

There was large fall in solubility and ATPase activity in shrimp, delayed by 8-12 hrs prior to icing.

The aerobic plate count (APC) increased considerably in the sample kept at ambient temperature for longer period prior to icing. Shrimp samples iced at 4, 8, 12 hrs delayed dominantly mostly the *Micrococcus*

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