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Research Article

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Comparing two methods of enumeration of bacteria

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Abstract The objective of this work was to count some microorganisms on agar petri dishes and Petrifilm in order to test the reliability of these two counting methods. The germs sought were coliforms and aerobic mesophiles. Seeding by incorporation into the mass on a petri dish and seeding on a petrifilm were used to count these germs in order to compare these two means of counting. The results showed that whatever the culture medium of this study, the number of colonies obtained on petrilms is higher than those obtained on petri dishes. Thus the microbial load in aerobic mesophiles obtained after enumeration on the petrifilm plate is $1.3\pm0.7x\ 10^8$ CFU/g and that obtained after enumeration on a petri dish is $1\pm\ 0.3x10^6$ CFU/g. The total and fecal coliform loads obtained after counting on the petrifilm plate are respectively $1.1\pm\ 0.5\ x\ 10^7$ CFU/g and $6.9\pm3.1\ x\ 10^5$ CFU/g. which are higher than the total and fecal coliform loads obtained after counting on petri dishes with respective loads of $7.9\ \pm\ 2.3\ x10^6$ CFU/g and $5.8\pm\ 1.7x10^5$ CFU/g. Although the use of petrifilm gives accurate results more quickly but they are expensive on the other hand petri dishes are inexpensive and can also be used to transplant colonies and make streaks to have well isolated colonies.

Keywords Petri dish, Petrifilm, Coliforms, Aerobic mesophiles, Enumeration

Introduction

The objective of microbiological food analysis is to control the marketability of food and hygienic quality. For this control, there are several bacteriological methods of enumeration for the search and identification of all microorganisms. The purpose of this enumeration is to determine the number of microorganisms belonging to a given set in a given volume either in liquid or solid medium [1]. Some of the most well-known enumeration techniques include petri dish agar enumeration, tube agar enumeration and petrifilm enumeration. In fact, the preparation of the culture medium for obtaining the agar on a petri dish is laborious and time-consuming. First of all, the enumeration agar must be prepared according to the manufacturer's standards and recommendations. This is done after weighing a few grams of the powdered medium in a well defined volume of distilled water and homogenizing it. The medium is then boiled until completely dissolved and sterilized in an autoclave at 121°C for 15 minutes. The medium is allowed to cool to a temperature of 40 to 45°C before being poured into a tube or petri dish [2]. In addition, Petrifilm^{MC} plates, which are bacterial culture plates containing a dry medium and a cold water-soluble gelling agent, are ready-to-use products developed by 3M (St. Paul, MN). These films are covered with culture media, so there is no need to prepare the media and savings in manpower and time are achieved [3]. However, the most commonly used methods in studies for the enumeration of microorganisms are traditional methods (agar on petri dish, tube medium). Thus the objective of this work is to count some microorganisms in cassava ferment using the petri dish and Petrifilm counting techniques in order to test the reliability of these enumeration methods.

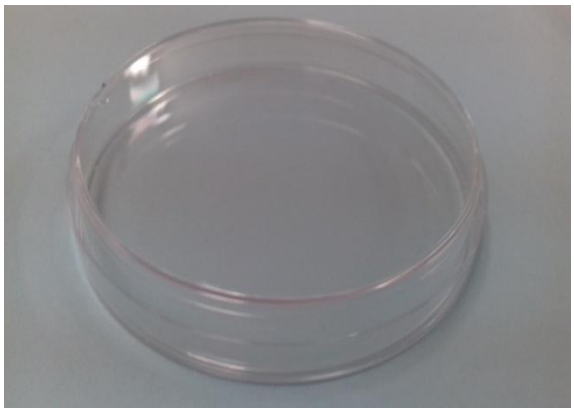
Materials and Methods

Materials

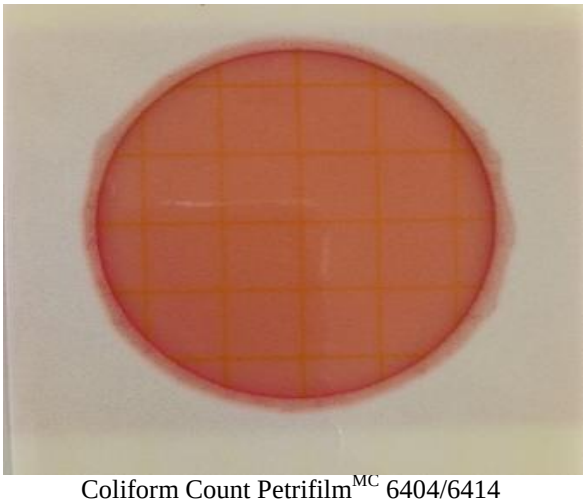
Biological Material

Figure 1: Traditional cassava inocula

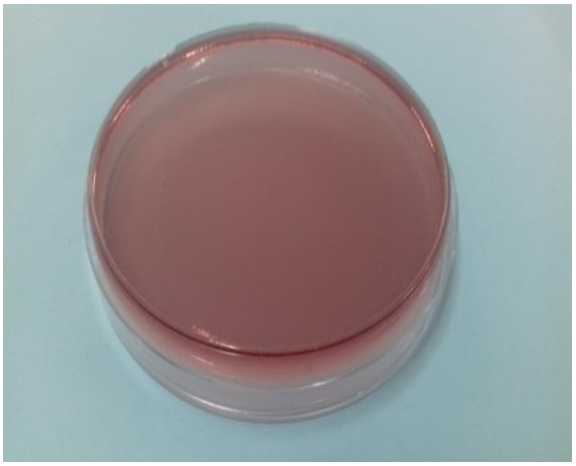
Technical Materials



Empty petri dish

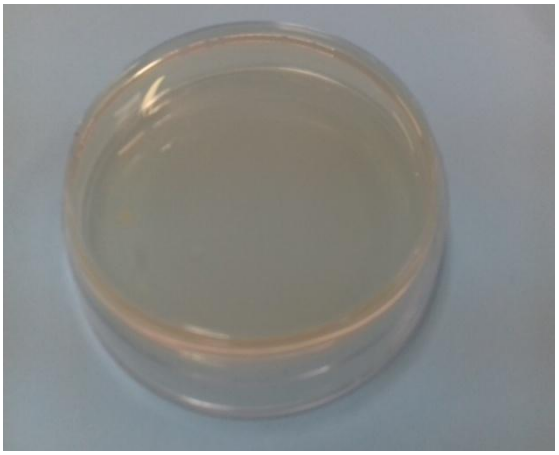


Coliform Count Petrifilm^{MC} 6404/6414



Petri dish containing VRBL agar for coliform enumeration

Aerobic mesophiles Count Petrifilm^{MC} 6400/6406



Petri dish containing PCA agar for AM enumeration

Figure 2: Technical materials, VRBL: Violet Red Bile Lactose agar, PCA: Plate Count Agar, AM: Aerobic mesophiles

Methods

Samples: The samples of traditional cassava inocula were taken with women producers of attieke in Abidjan, Côte d'Ivoire and transported in an icebox directly to the laboratory for analyses

Description and use of petri dishes

Petri dishes are small flat-bottomed boxes, made of transparent or plastic glass, round or square, with a diameter of a few centimetres (usually between 5 and 20). They consist of two halves, a cover and a bottom, which are embedded in each other, making them impervious to suspended elements, even small ones. The contents are thus protected from possible contamination by bacteria in the air, while allowing the gases produced by the bacteria to escape into the box. They have the advantage of being easy to handle, stackable and inexpensive [4]. The preparation of both media is done after weighing a few grams of the powdered medium in a well defined volume of distilled water and homogenizing it. The medium is then boiled until completely dissolved and sterilized in an autoclave at 121°C for 15 minutes. The medium is allowed to cool to a temperature of 40 to 45°C before being poured onto a petri dish for surface spreading seeding. For inoculations by incorporation into the mass, after the various dilutions, 1 mL of each retained dilution is taken aseptically and poured with a first layer of.15 to 18 mL of previously melted PCA or VRBL and reduced to 45-50°C in a petri dish. The inoculum and culture medium are well homogenized by circular movements of the hand, in one direction and then in the other. After solidification, a second 5ml layer is added; then the Petri dishes are incubated, turned the lid down [2].

Description and use of Petrifilm ^{MC} Plates

Petrifilm ^{MC} Plates are ready-to-use products developed by 3M (St. Paul, MN). Films are covered with culture media, so it is not necessary to prepare the media and savings in manpower and time are achieved. Bacteria can be enumerated using Petrifilm ^{MC} enumeration plates. Bacterial culture plates containing a dry medium and a cold water-soluble gelling agent are used. 1 ml samples are added directly to the plates. A light pressure is applied to the top film with the plastic diffuser to spread the sample over 20 cm^2. After allowing the gelling agent to solidify, the plates are then incubated and counted. Validation and collaborative studies have shown that the Petrifilm ^{MC} AC Plate method does not differ significantly from traditional methods [3].

Microbiological analysis

Preparation of stock solutions, inoculation of agar plates and petrifilms, cultivation and quantification of microorganisms were carried out according to [5]. For all determinations, 10 g of the samples were homogenized in a stomacher with 90 ml of sterile peptone buffered water (AES Laboratoire, COMBOURG France). Tenfold serial dilutions of stomacher fluid were prepared and spreadplated for determination of

microorganism counts. Enumeration of total and faecal coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany) which were incubated for 24 h at 30 °C for total coliforms and 44 °C for faecal coliforms. Aerobic mesophiles were enumerated on plates of Plate Count Agar (PCA Oxoïd LTD, Basingstore, Hamsphire, UK) and incubated at 30 °C for 2 days.

Statistical Analysis

Software R. 3-01, ANOVA method with Duncan post-hoc test, significance level 5% was used. This software made it possible to calculate the means, the standard deviations of the microbiological parameters. It also made it possible to compare the means of the microbiological parameters of the samples and to determine whether the differences observed in the means of the microbiological parameters are significant at the 5% threshold

Results and Discussion

Microorganisms can be counted using Petrifilm^{MC} plates and petri dishes. However, bacterial colonies develop more on petrifilms than on petri dishes as shown in Figure 3.

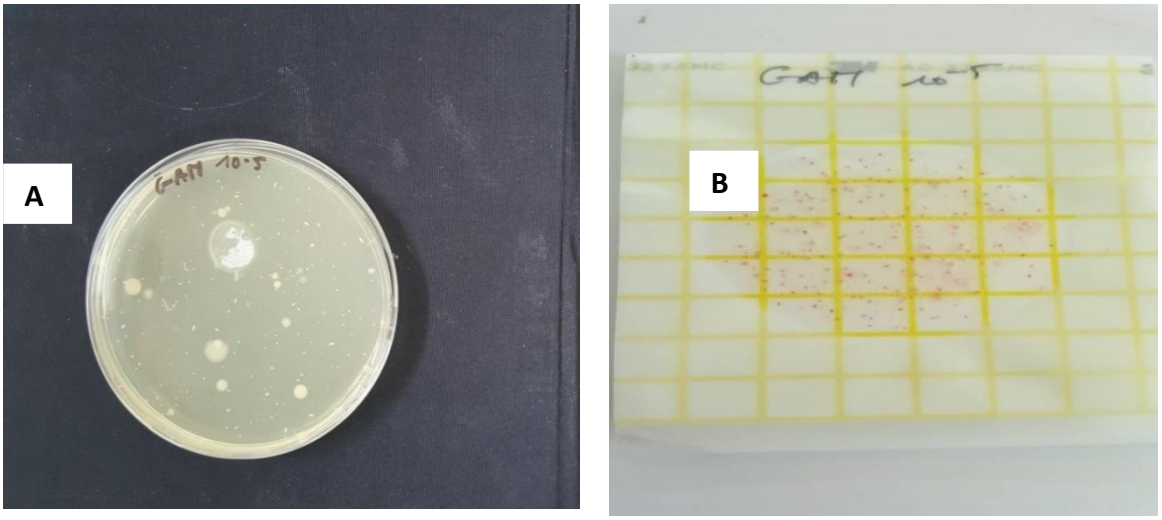


Figure 3: Colonies of aerobic mesophilic germs observed on petri dishes and on Petrifilm Plate, A: petri dishes, B: Petrifilm Plate

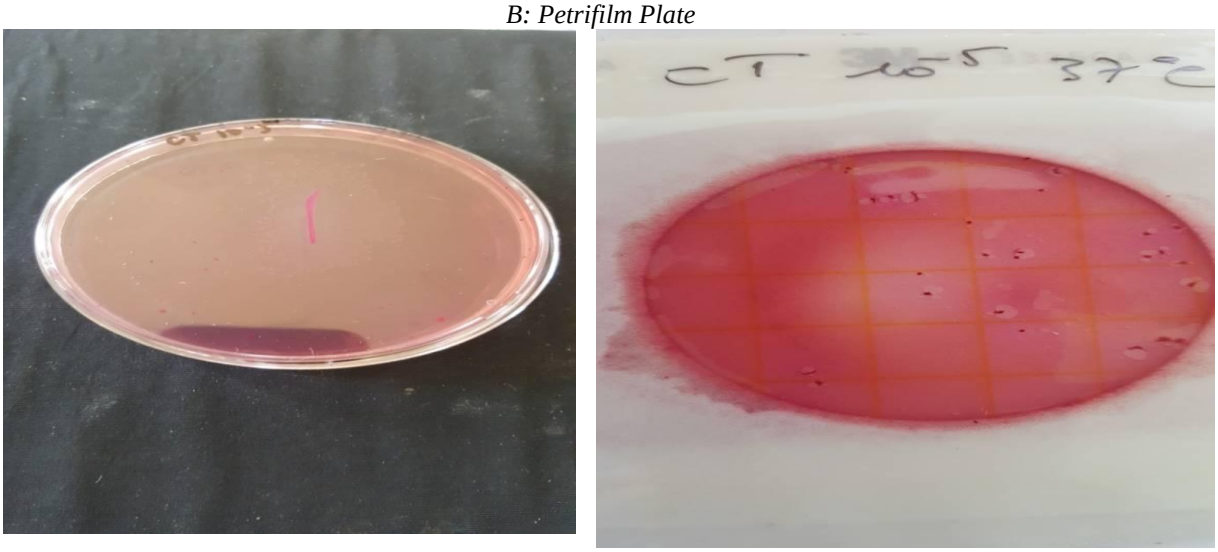


Figure 4: Colonies of Coliforms observed on petri dishes and on Petrifilm Plate, A: petri dishes, B: Petrifilm Plate

Regardless of the culture medium in this study, the number of colonies obtained on Petrifilm is higher than those obtained on petri dishes. GAM colonies obtained at 10^{-5} dilution on the Aerobic Count (AC) Petrifilm^{MC} plate 6400/6406 are more numerous than those obtained at the same dilution on the PCA petri dish medium.

Under dilutional conditions, the colonies recorded on the Petrifilm^{MC} Coliform Count Plate 6404/6414 are many as those obtained on the VRBL medium in a petri dish (Figure 4).

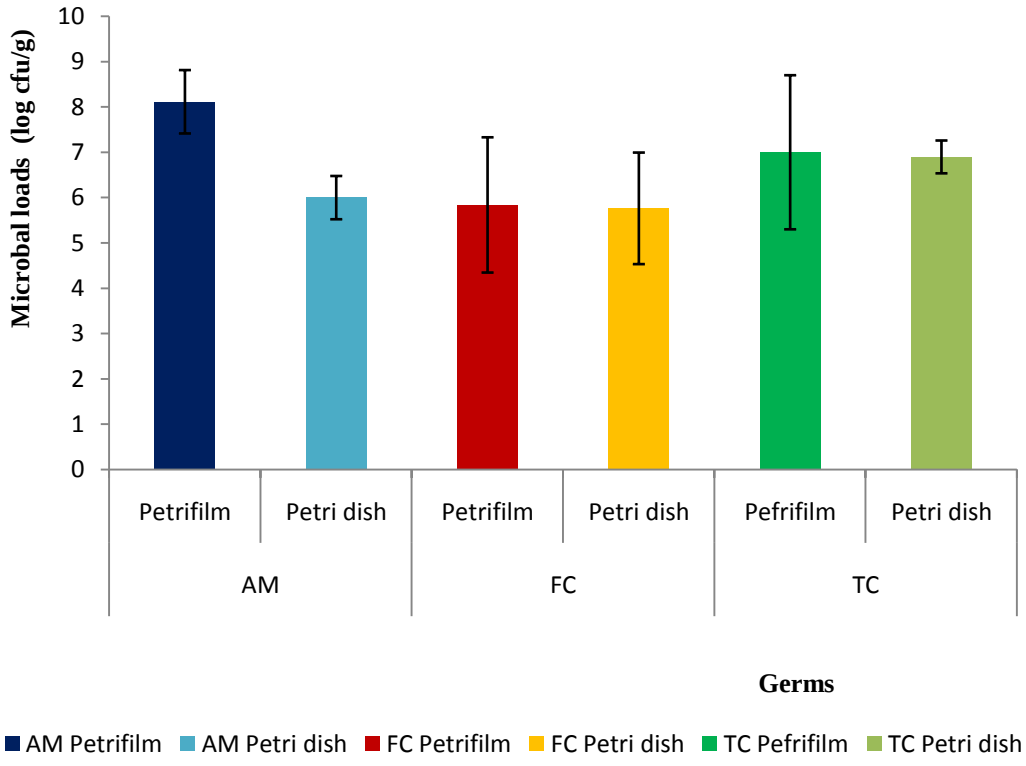


Figure 5: Microbial loads of aerobic mesophilic germs and Coliforms obtained from the petri dish and Petrifilm enumeration methods; AM: aerobic mesophilic, FC: fecal coliforms, TF:total coliforms

This indicates that the use of petrifilms for bacteria enumeration gives more effective results than the use of petri dishes. Indeed, Petrifilm^{MC} plates, which are ready-to-use products, help reduce media loss, simplify the analytical process and provide accurate results more quickly [6]. On the other hand, the use of petri dishes is laborious and long. First of all, the enumeration agar must be prepared according to the manufacturer's standards and recommendations but also recommends more attention to hygiene and sterilization in order to avoid contamination of the culture [2]. Thus the microbial load in aerobic mesophilic germs obtained after enumeration on the petrifilm plate is $1.3\pm0.7 \times 10^8$ CFU/g and that obtained after enumeration on a petri dish is $1\pm 0.3 \times 10^6$ CFU/g. The total and fecal coliform loads obtained after counting on the petrifilm plate are $1.1\pm 0.5 \times 10^7$ CFU/g and $6.9\pm3.1 \times 10^5$ CFU/g respectively, which are higher than the total and fecal coliform loads obtained after counting on a petri dish with respective loads of $7.9 \pm 2.3 \times 10^6$ CFU/g and $5.8\pm 1.7 \times 10^5$ CFU/g (Figure 5). The differences in microbial load obtained on petri dishes and petrifilms are not significant at the 5% threshold.

This result is consistent with that of [3], which indicates that validation and collaborative studies have shown that the Petrifilm^{MC} AC Plate method does not differ significantly from traditional methods. Although the use of petrifilm gives accurate results more quickly, petrifilm is expensive, but petri dishes are easily handled, stackable and inexpensive. In addition to enumeration, the boxes can be used not only to transplant colonies and make streaks to obtain well isolated colonies, but also because of their transparent, flat, inexpensive and evaporation-preventing characteristics, making them particularly suitable for observing small organic or inorganic elements [4]. Otherwise, samples contained aerobic mesophiles and coliforms. The presence of such microorganisms in traditional inocula cassava could be due contamination by the contact of the product with air, the product handling and the plastic bag used and lake of hygiene practices during packaging [7].

Conclusion

Mass incorporation seeding on petri dishes and petrifilm seeding were used for the enumeration of aerobic mesophiles and coliforms in order to compare these two means of enumeration. Regardless of the enumeration culture medium, the number of colonies obtained on petrilms is higher than those obtained on petri dishes. The use of petrifilm seems to give more accurate results than sowing by incorporation into the mass on a petri dish. However, in addition to counting, petri dishes can be used not only to transplant colonies and make streaks to have well isolated colonies. It would therefore be to make petrifilms capable of being used to transplant colonies and make streaks.

Acknowledgements

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