Although oospores play a significant role in sexual reproduction, the sexual recombinant of the pathogen and the primary source of inoculum in the field, but little information is reported about the influence of some factors which stimulate the formation, germination and the pathogenicity of their progeny. Thus it is an important precedent for research in P. capsici, especially on black pepper.

REFERENCES


Traditional Nata fermentation process is not yet fully studied nor comprehended at the molecular level, although the process has enough reason to be explored. Randazzo et al. (2002), have studied community activity and the dynamics of bacterial populations during cheese production. Ampedatto et al. (2001), also studied the "Scour Cassava" fermentation process and they found that the dynamics of microorganisms were sequentially changed during the fermentation process. We consider that if we studied the dynamics of the microbial populations, we could have better understanding on the interactions of microorganisms in the fermentation processes so that we can systematically establish a consistent Nata starter culture. Population dynamics need to be studied through community analysis because it represents an excellent approach to comprehending the function and survival of a community. This analysis gives the opportunity to identify dominant and unique strains in a controlled environment (Marsh et al. 2000). Giraffa and Nevanlinna 2001, reported that the first step to comprehend these concepts in food microbiology is to analyze microbiological profiles and community structures and, also their function in the environment and biologic conditions in food.

The challenge is the difficulty in cultivating microorganisms from food on laboratory standard media since most microorganisms from nature are not yet cultivable. (Giraffa and Nevanlinna 2001). Ampedatto et al. (1999), have compared the standard microbiological techniques, and techniques that do not depend on cultivated processes, to examine microbial populations. They found that the culture-independent technique is the most suitable to depict population dynamics. Wieland et al. 1991, found that amplification of the 16S-RNA gene, cloning and sequencing it represents one of the important methods to identify microorganisms directly from nature (culture-independent). This technique is essential in studying the dynamics of a microbial population in Nata fermentation due to the extreme pH of media via the acid cultivation conditions (pH 2-3). Our previous investigations indicated that all of the bacteria involved in Nata fermentation could be cultured. This research aims to study the diversity and dynamics of the bacterial population during traditional Nata de Coco fermentation.

MATERIALS AND METHODS

Natala media solution from the fermentation processes with the Badan and Good outcomes were sampled at days 5, 6, 7, 8, 9, and 11 for ARDRA. Natala media solution was categorized as "Good" if it contained Nata with a thick and smooth texture. In contrast, a Natala media solution was categorized as "Bad" if it yielded Nata with the hard texture that had bubbles of gas trapped in it. One of the A. xylimum collection strains B1 was marked by molecular marker to become A. xylimum Strain B1-NaR. This was used to analyse the growth of A. xylimum and the viability of other bacteria in fermentation media. Samples of media and strains of A. xylimum used in this study were collected from a Nata de Coco company in Jakarta.

Analysis with Acetobacter xylimum Strain B1-[B], which is initially sensitive to nalidixic acid, was screened for spontaneous nalidixic-resistant mutates by cultivating it repeatedly at media supplemented with that antibiotic. A. xylimum...
Strain IB-1 NaI-R, which is resistant to nalidixic acid, was subsequently grown in media without antibiotic supplementation as control (MedS), the medium with the addition of nalidixic acid (Nal), and the heat-treated medium by boiling at 100 min (Ee) to eliminate most vegetative bacterial cells. Bacteria populations grown in different treatments were examined on the first, fifth and tenth days of cultivation. Media used for the growth were as described previously (Marchesi et al., 2005).

Amplification and Cloning of the 16S rRNA Gene. Amplification of the 16S rRNA gene was conducted employing f3′: 5′-CAGGGCTACAACATTGAAGTC-3′ and e3′: 5′-GGCTCAGGCTGACGGCC-3′ for the Bacteria Domain group (Moffett et al., 1990). 16S rRNA amplicons were purified employing Wizard SV Gel and the PCR Clean-Up System. The purified DNAs were ligated into pGEM-T Easy vector (Promega, Madison, WI, USA) and transformed into E. coli DH5α (Invitrogen, Carlsbad, CA, USA). Transformants were selected on Luria Agar media (LA)=Ampicillin (10 μg/ml) supplemented with X-gal (40 μg/ml). 16S rRNA gene in recombinant plasmid library (collection of 16S rRNA genes in pGEM-T vector) were amplified again using M13F and M13R primer (Moffett et al., 2000) to obtain individual 16S rRNA genes. This step would ensure that the amplified 16S rRNA genes were recombinant plasmids and not from the bacterial host 16S rRNA gene.

Amplified Ribosomal DNA Restriction Analysis (ARDRA). The 16S rRNA gene, amplified from recombinant plasmids was digested with restriction enzymes RsaI or HpaII, to yield a specific pattern representative of the existing bacteria and designated as Profile 1. Profile 2, etc. The percentage of specific patterns calculated each day of fermentation and was depicted as a population dynamic curve.

**RESULT**

**Growth of A. xylinum in the Presence of Microbial Community.** Referring to the obtained data, we made a cell growth histogram depicting the growth profiles of A. xylinum Strain IB-1 NaI-R in fermentation media with different treatments (Fig 1). Fig 1 indicates that fermentation media with a blanching treatment (Bic) did not enhance the growth of Strain IB-1 NaI-R compared to that of the control medium (Nata fermentation medium without any treatment). In the fermentation medium with nalidixic acid supplementation, the growth of IB-1 NaI-R became very depressed. Although the population did increase, the sum of the cell count was as high as that in the control medium or blanching treatment. This result suggested that the pre-existing bacterial population in the media were essential for successful Nata fermentation and might have positive or synergistic effect to the growth of Strain IB-1 NaI-R.

ARDRA Reveals Bacterial Profiles During Nata Fermentation. Results of ARDRA analysis showed the existence of at least twenty different bacterial group during Nata fermentation (Fig 2). Each ARDRA profile found in Nata fermentation was calculated as a percentage to the total profiles every day starting from the fifth day up until eleventh day. Five profiles were considered to be unique because their presence could only be found over certain specific days of fermentation (Fig 3). Unique ARDRA profiles include profile 1 to 5. Profile 6 to 22 was not depicted in a growth curve because we found them only on certain days and they did not show significant percentage numbers of the total population (data is not presented in this article).

**DISCUSSION**

A. xylinum with antibiotic resistance marker was employed, in the laboratory scale, to examine the influence some media treatment on the growth of A. xylinum during Nata fermentation. Three kinds of media were used, i.e., a media without treatment, media with blanching for 10 min to eliminate as many as possible contaminants in the LA and media with nalidixic acid supplementation (20 μg/ml) to suppress the growth of other bacteria sensitive to this antibiotic. The growth of contaminants was expected to be a pressing give enable to A. xylinum to "outcompete" a yield and produce good Nata gel. Strain IB-1 was marked for purpose of cell estimation when they were grown in a 0.1 ml Nata fermentation. As we assumed that Strain IB-1 NaI-R could be maintained as dominant population, this isolate will grow fast and produce excellent Nata gel.

The results showed that Nata which was produced during the fermentation process, when the natural contaminating population was suppressed with either a blanching treatment or nalidixic acid supplementation, was inferior in quality compared to that of the control media. Therefore, the presence of foreign bacteria at the control media might have a synergistic effect and stimulate rapid growth of the A. xylinum population. In traditional Nata fermentation, media sterilization was often conducted under sterile conditions. Ho...
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percentage of specific patterns calculated on each day of fermentation and was depicted as a population dynamic curve.

**RESULT**

Growth of *A. xylinum* in the Presence of Microbial community. Referring to the obtained data, we made a cell growth histogram depicting the growth profiles of *A. xylinum* rain IB-1-Nal-R in fermentation media with different treatments (Fig. 1). Fig 1 indicates that fermentation media with aanching treatment (Bic) did not enhance the growth of rain IB-1-Nal-R compared to that of the control medium (data fermentation medium without any treatment). In the fermentation medium with nalidixic supplementation, the number of IB-1-Nal-R became very depressed. Although the population did increase, the sum of the cell count was not as high as that in the control medium or blanching treatment. The result suggested that the pre-existing bacterial population in the media was essential for successful Nata fermentation and might have positive or synergistic effect to the growth of IB-1-Nal-R.

ARDRA Reveals Bacterial Profiles During Nata Fermentation. Results of ARDRA analysis showed the existence of at least twenty two different bacterial group during its fermentation (Fig. 2). Each ARDRA profile identified in Nata fermentation was calculated as a percentage to the total profiles every day starting from the fifth day up until eleventh day. Five profiles were considered to be unique because their presence could only be found over certain specific days of fermentation (Fig 3). Unique ARDRA profiles include profiles 1 to 5. Profile 6 to 22 was not depicted in a growth curve because we found them only on certain days and they did not show significant percentage numbers of the total population (data is not presented at this article).

**DISCUSSION**

*A. xylinum* with antibiotic resistance marker was employed in the laboratory scale, to examine the influence of some media treatment on the growth of *A. xylinum* during Nata fermentation. Three kinds of media were used, i.e. media without treatment, media with blanching for 10 minutes to eliminate as many as possible contaminants in the media, and media with nalidixic acid supplementation (20 μg/ml) to suppress the growth of other bacteria sensitive to this antibiotic. The growth of contaminants was expected to be suppressed to give enable to *A. xylinum* to 'outcompete' and yield population of good Nata strain. Strain IB-1-Nal-R was used for the purpose of cell estimation when they were re-grown on media with nalidixic acid supplementation. We assumed that if Strain IB-1-Nal-R could be maintained as dominant population, this isolate will grow fast and produce excellent Nata gel.

The results showed that Nata which was produced during the fermentation process, when the natural contaminant population was suppressed with either a blanching treatment or by nalidixic acid supplementation, was inferior in quality compared to that of the control media. Therefore, the presence of foreign bacteria at the control media might have a synergistic effect and stimulate rapid growth of the *A. xylinum* population. In traditional Nata fermentation, media preparation was often conducted under sterile conditions. How ever, the fermentation did not always fail or result in 'Bad Nata'. This might explain why the process existing in the media preparation and also during the fermentation process, could enhance Nata production and might possibly show exhibit symbiosis or excrete essential factors required for cellulose biosynthesis.

In this study we define 'Good Nata' fermentation as one which will generate a thick (1.5–2 cm), homogenous cellulose gel with high transparency, while 'Bad Nata' fermentation will generate frothy thin (frequently less than 0.5 cm), soft with white or opaque color Nata gel after 8 days of fermentation (Sekahuru 2005).

In this study, ARDRA was employed to better understand the bacterial community involved in the production of 'Bad' and 'Good Nata'. This analysis was based on direct extraction of total DNA from both cultured and uncultured bacteria. Specific bacterial strain in the Bacteria Domain could be identified by their specific profiles generated from the electrophoregrams of 16S rDNA and eDNA digested with restriction enzymes (HaeIII or RsaI).

Results of the ARDRA indicated that in a traditional Nata fermentation process, *A. xylinum* represented the dominant species during a process which could also have a symbiosis, or association with other bacteria present in either coconut water or coconut milk that might generate a mutual effect in 'Good Nata' production or an antagonistic effect in 'Bad Nata' production. Bacterial growth pattern shown in Fig 3, indicated a sharp fluctuation for profile 2, 3, 4, or 5 during the course of fermentation. In fermentation generating the 'Bad Nata', this
growth profile was very erratic, while at fermentation with a good outcome, this type of profile was less erratic and tended to stabilize over time. Profile I did not show any difference of their population dynamics either in the ‘Bad’ or ‘Good Nata’ production. Profile 2 in ‘Bad’ fermentation tends to show fluctuation while in good fermentation it tends to stabilize with a low percentage of variability. On the other hand, profile 4 in both ‘Good’ and ‘Bad’ fermentation did not show the existence of different population dynamics.

This fermentation process showed the existence of unique profiles, i.e., profile 3 and 5 from the Bacteria Domain. Profile 3 showed rather sharp difference between ‘Bad’ and ‘Good Nata’ in a fermentation resulting in ‘Bad Nata’, this profile tend to fluctuate and rise in the final fermentation process. On the other hand, in fermentation with the ‘Good Nata’ the existence of this profile tended to be minimal or was not visible. Profile 5 on fifth day of the fermentation process for the ‘bad’ result showed the highest percentage (70%), while on the same day this profile showed only 25% in a fermentation process until yielded the ‘Good Nata’. In a fermentation process with the ‘bad’ result, this pattern was not detected on subsequent day, while, for the fermentation process with a ‘good’ result, this pattern was still detectable in spite of its low amount (5%) on eleventh day. We conclude that community unique profiles represent one of the key factor which in this study can be considered essential indicators for ‘Bad’ or ‘Good Nata’ fermentation. This analysis could be more dramatic if the sampling had not been limited to begin from day 5 where cellulose pellets start to emerge.

Another unique matter is the amount of ‘other’ profiles present is relative small number and the flat spreading during the fermentation process with the ‘good’ result as compared to fermentation process yielding the ‘bad’ result (data not presented). A possible explanation for the existence of different type of bacteria at different step represents a symbiosis process, where a different set of bacteria are required at different steps of fermentation to provide essential nutrients to A. xylinum for its cellulose biosynthesis. The presence of these bacteria could supply otherwise deficient but essential nutrients which are important for the growth of A. xylinum for ‘Good Nata’ production. This result also indicates that traditional Nata fermentation, which tends to be semi-aseptic, might be required to provide some beneficial bacterial inocula for ‘Good Nata’ production (Fig. 1) since A. xylinum grow better in medium without antibiotic supplementation or having blanching treatment.

Other factors which might have an effect to bacterium population dynamics is the change of pH of the fermentation medium during the process. At the start of fermentation, the pH of the media is 3.9. This value dropped over time until it reached approximately pH 2.0 at the end of the fermentation. This might have effect on the complexity of the bacterial community profiles.

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