Single Spore Isolation as a Simple and Efficient Technique to obtain fungal pure culture

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Abstract: The successful identification of fungi by phenotypic methods or molecular technique depends mainly on the using an advanced technique for purifying the isolates. The most efficient is the single spore technique due to the simple requirements and the efficiency in preventing the contamination by yeast, mites or bacteria. The method described in the present work is depends on the using of a light microscope to transfer one spore into a new culture medium. The present work describes a simple and efficient procedure for single spore isolation to purify of fungi recovered from the clinical wastes.

1. Introduction

One of the most important steps in the identification or application of fungal species in the biotechnology is to confirm that the fungal isolates used are pure. Different techniques have been used for the purification of fungi including the direct subculture of the fungal isolate onto a new medium. However, the studies revealed that this method is not accurate because in many of cases the researchers failed to identify the fungal isolate correctly [1]. In the biotechnology and industrial activities using a pure culture of fungal species represent critical step to achieve the target required. In the industry the contamination of pure culture might lead to destroy of the final products or produce secondary undesirable products. Single spore isolation is one of the best procedures in order to get a pure culture of the fungi. The procedure for the technique has been described by several authors [2,3,4]. However, these the procedure described need well trained technicians and more facilities to carry out successfully. Therefore, the present study aimed to provide a simple and efficient procedure steps for single spore isolation to be more applicable for researchers and students without the need of specific instruments.

2. Methodology

2.1 Clinical wastes sampling

The clinical wastes samples were obtained from a Wellness Centre at University Sciences Malaysia (USM), Penang Malaysia. The samples were collected in the biohazards clinical waste bags, kept inside a polystyrene box with ice and transported to the pathological laboratory [5] (Fig. 1). The samples were

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segregated into five groups and subjected to the microbiological analyses immediately under aseptic conditions.

2.2 Recovery of fungi on the culture medium

The direct plate technique was used for culture of fungal isolates from the samples on the surface of V8 juice agar medium (V8A). The medium was prepared by mixing V8 juice (163 mL), Agar (4 g) and CaCO3 (2 g) in distilled water (87 mL) to make 250 mL with pH 5 adjusted with HCl (0.1 N) and NaOH (0.1 M) [1,6]. The homogenized medium by a magnetic stirrer was autoclaved at 121 ˚C for 20 min. A fixed volume of (1 mL) chloramphenicol (500 mg L^{-1}) was mixed with the medium (after cooled to 50˚C) to prevent the bacterial contamination. The medium was poured immediately into Petri plates.

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Figure 1. Sampling of the clinical wastes from USM Wellness Centre; **A)** Syringe waste; **B)** lancet waste; **C)** Gloves waste.

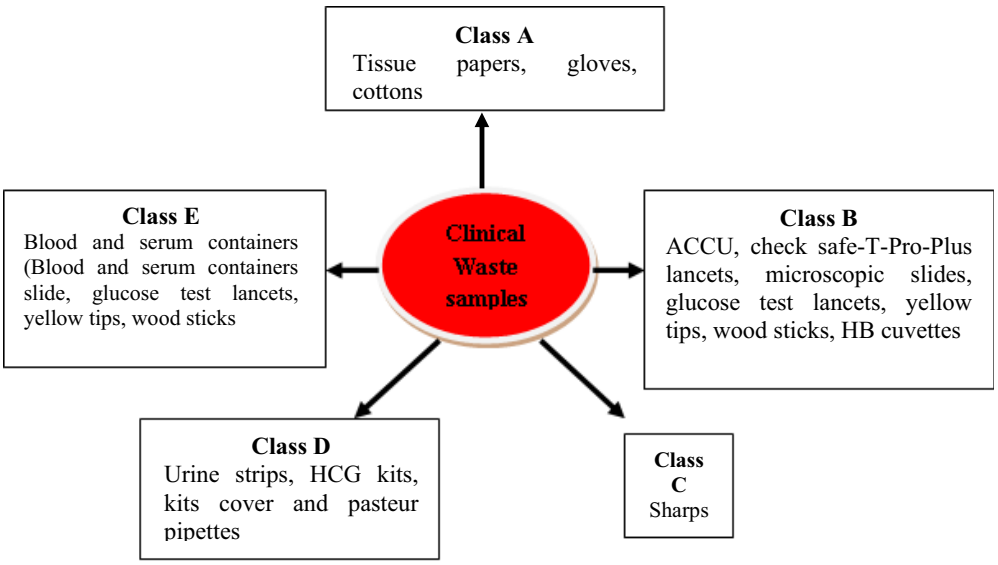

Figure 2. Clinical wastes generated at Wellness Centre of USM (Main Campus)

In order to get the fungal culture on V8A, 2-3 pieces (1.5×1.5 cm) of Class A samples, while pieces of B, C,D and E samples and 0.1 mL of liquid waste sample was used. For the fungal spores from the storage room, a new V8A medium was placed in the storage room for 60 min. The plates were incubated for 7 to 12 days at 28˚C.

2.3 Single spore technique

The single spore technique was conducted in the laboratory as follows; the fungal isolate grown on V8A was sub-cultured onto a new V8A medium and incubated for 5 days, the plate was placed under stereo light microscope. An inoculation with 100-500 of fungal spores was transported onto potato dextrose agar (PDA) medium (the medium was prepared two days before and dried at 28 ˚C for 24 h to facilitate absorbance excess water film) by sterilized scalpel. The inoculation was spread by glass spreaders on the surface of the medium using 0.1 mL sterilized distilled water (SDW). The plates were left inside laminar flow for 10 min to dry. The inoculated media were incubated at 28˚C for 16-18 hours. Thereafter, one germinated spore was selected and transformed into new PDA medium. The fungal growth was recorded after 7 days of the incubation at 28˚C. All the septs were performed as recommended by Choi et al. [3] to minimize the contamination of pure cultures by mites were performed carefully.

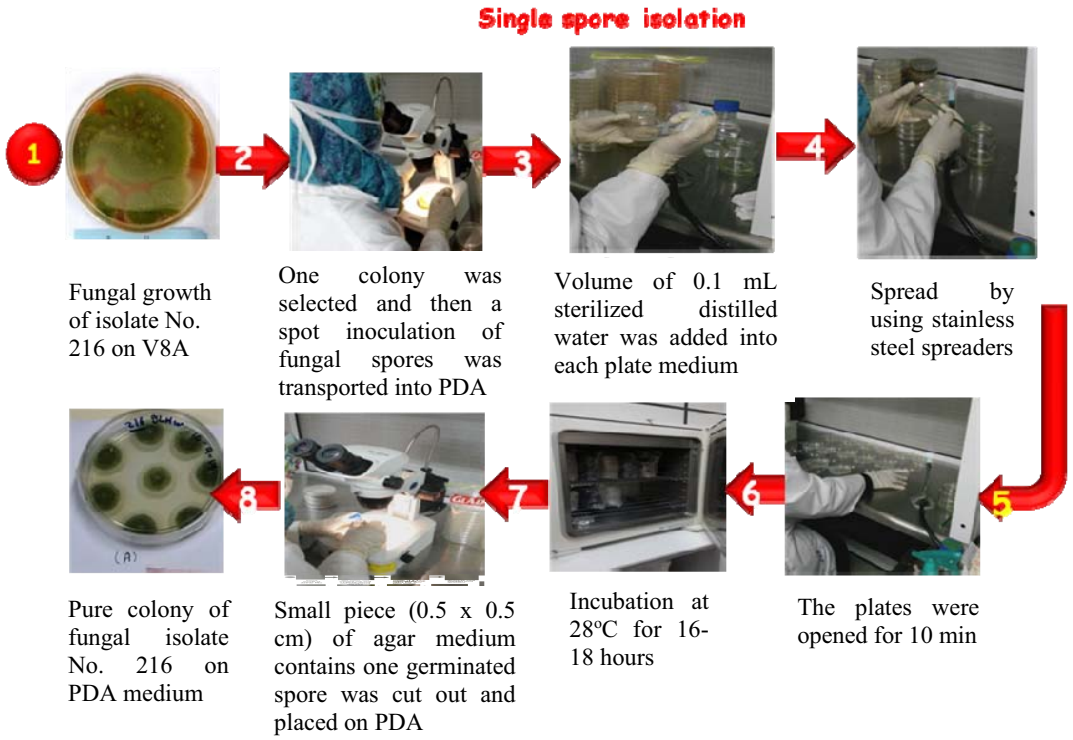

Figure 3. Procedure of single spore isolation

3. Results and Discussion

3.1 Fungal isolates obtained from the samples

The fungal growth on the V8A medium after the incubation period is depicted in Figure (4). The medium was selected among different culture media because it exhibited high efficiency to recovery of fungi from the samples [1] Moreover, V8A is an enrichment medium has the necessary composition which enhance the fungi growth and sporulation [3]. However, the disadvantages of this medium include the low efficiency in the occurrence of reverse colour of the fungal colony. Hence, V8A might be used for the first isolation of fungal from the samples, while the culture characteristics might be described on the others media.

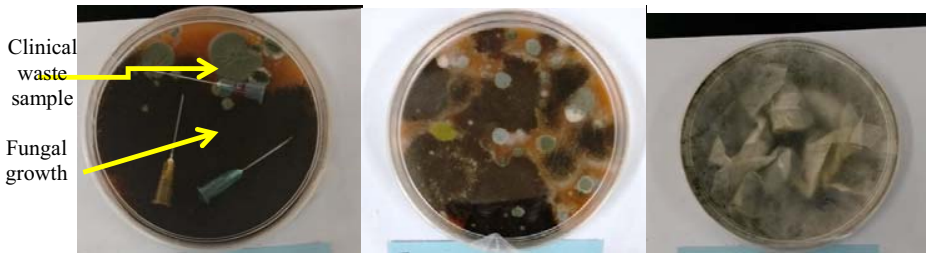

Figure 4. First isolation of fungi from clinical wastes on V8A medium, A) Syringe sample, B) Airborne fungi from storage room; C) tissue sample

3.2 Purification of fungal isolates by single spore technique

In order to successful identification of fungi, an accurate purification method is required. Hence, the single spore isolation method was conducted in the current work. This method is among different types of the purification techniques which has high efficiency to get a very pure culture of the fungi and then correct identification by phenotypic methods [3,7,8]. The results in the present work revealed that the fungal colony purified with single spore method appeared with a better occurrence compared to that obtained by using the needle (Fig. 5). The explanation for these results is belonged to the principle of single spore technique which rely on the selection only one spore and then transferred it into a new medium. In comparison the using of the needle for the subculture or purification many of the spores are transmitted and subculture, therefore, the colonies imbricated together. One more advantage of single spore isolation is the morphology of the colony on the culture medium, where it can get the colony diameter more easily than that purified by direct isolation. Moreover, most of the fungal colonies raised from the single spore has a circular shape while it is irregular in the shape when it is cultured by direct isolation. Fig. 5 shows the example of fungal isolate sub-cultured by single spore technique and direct technique. The single spore isolation was also efficient for the purification of fungal isolates which have failed for sporulation on the culture medium (Fig. 6), in this case one hyphae of the fungal isolates was used in a replacement for one spore.

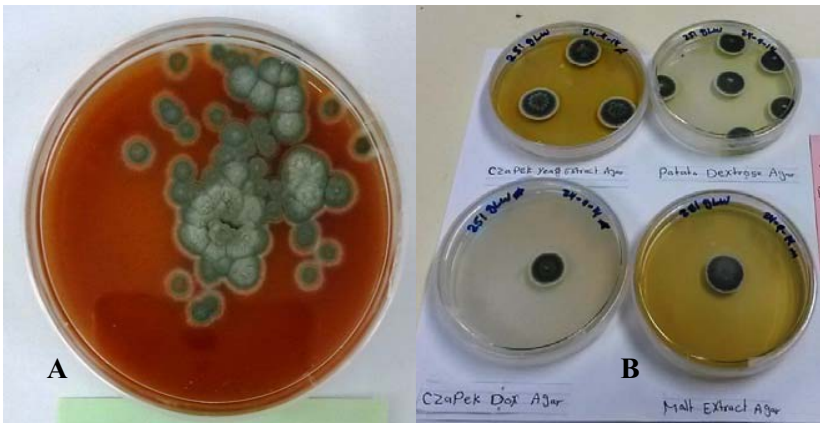

Figure 5. *P. citrinum* sub-cultured on culture media; A**)** Direct isolation by needle on V8A; B) Single spore isolation on different culture medium

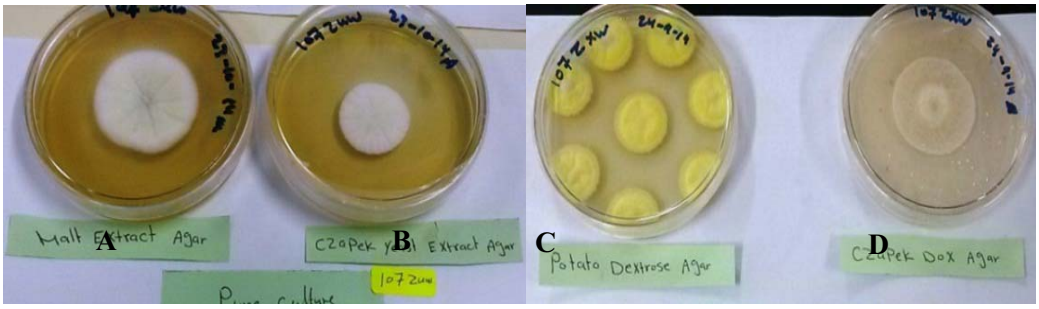

Figure 6. Fungal isolate No. 107ZXW (non spore production) on different culture media purified by single spore isolation A) MEA; B) CYA; C) PDA; D) CZ media

4. Conclusion

It can be concluded that the single spore isolation procedure described in the present study was efficient to obtain pure culture of fungi from clinical wastes in comparison to the traditional direct subculture method. Therefore, it can be used without the need for specific instrument or well-trained technicians.

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