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Variations and Hybridization Compatibility of Single Basidiospore Isolates of *Pleurotus sajor-caju* (Fr.) Sings

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ABSTRACT

Pleurotus sajor-caju (Fr.) Sings, a mushroom of the family Pleurotaceae, is gaining popularity due to its high nutrient content and capability of growing on various agricultural wastes. There is a need to breed new strain of *P. sajor-caju* to meet the rising demands of the increasing human population. Strain improvement is achievable through selection and hybridization. Unfortunately, there is limited information regarding the genetic variations of *P. sajor caju* in Malaysia. Therefore, this study is of interest to document the morphological variations of single basidiospore isolates and to generate hybrids. A total of 200 single basidiospore isolates (SB) obtained from a commercialized strain of *P. sajor-caju* were obtained from local supermarket in Kuching, Sarawak, and cultured individually on potato dextrose agar. These 200 SBs were characterized morphologically and divided into three main groups based on colony morphology i.e. scattered, rough and smooth. Variations can still be observed in each main group. From each main group, SBs representing the variations were further categorized based on their colony diameter growth after 7 days of post inoculation (CD-7dpi), i.e. slow growing CD-7dpi (SGCD-7), medium fast growing CD-7dpi (MFGCD-7) and fast growing CD-7dpi (FGCD-7). Ten FGCD-7 and ten SGCD-7 isolates were selected for hybridization. The selected SBs were hybridized in all possible pairings without repetition. Sixteen hybridized isolates were recognized and characterized based on CD-7dpi. For all FGCD-7 pairings, SGCD-7 pairings, and between FGCD-7 and SGCD-7 pairings, hybridized isolates had higher CD-7dpi than at least one of its parents were identified. The new hybridized isolates are interesting materials for future study.

Keywords: *Pleurotus sajor-caju*, single basidiospore isolate variations, hybrid

INTRODUCTION

Mushrooms are becoming one of the main food sources which have acquired more attention particularly in the Asian countries (Rosli & Solihah, 2012). The cultivation of mushroom had taken place since prehistoric times especially in the eastern countries for their nutrient content and flavour (Sadler, 2003). The most widely cultivated mushrooms are from the genus *Pleurotus* or oyster mushrooms (Imran *et al.,* 2011).

Pleurotus sajor-caju, commonly known as Dhingri oyster or grey abalone oyster mushroom, is one of the well-known cultivated *Pleurotus* species. This species is currently gaining popularity, due to its nutrient contents (Schneider *et al.,* 2011; Pala *et al*., 2012; Rosli & Solihah, 2012). In addition, *P. sajor-caju* is reported to possess medicinal values such as preventing atherosclerosis (Schneider *et al.,* 2011), lowering cholesterol level and affecting glycemic response (Rosli & Solihah, 2012).

With the growing demand of *P. sajor-caju* and their huge acceptance for food products, there is a need for strain improvement. Strain improvement by conventional breeding can resolve these tasks. In order to develop new strain having desirable traits, the first step would be to generate and characterize the single basidiospore isolates. The presence of variations among the single basidiospore isolates is crucial for producing intra or interstrainal hybrids (Gupta *et al.,* 2011). However, the information on the range of morphological variations for single basidiospore isolates is insufficient for *P. sajor-caju.* This study attempted to isolate and characterize as much as possible single basidiospore isolates with different morphologies. Also in this study, hybridized isolates were produced by hybridizing selected single basidiospore and were characterized.

MATERIALS AND METHODS

Source of Spores and Single Basidiospore Strain Culture

A commercialized strain of *P. sajor-caju* was obtained from local supermarket in Kuching, Sarawak. Spore print of different basidiocarp were printed separately on clean papers. Each spore print was scrapped off using clean scalpel and put into Eppendorf tube separately which contain 1 ml of sterile distilled water forming a spore suspension. The concentration of spore suspension was adjusted to 50 spores/200 µL. A total of 200 µL spore suspension was spread on Petri dishes (⌀ 90 mm) containing potato dextrose agar (PDA) media using glass rod spreader. The plates were cultured in room temperature, with no light control. Germinated spores with non-overlapping mycelium were picked and transferred individually on PDA to obtained 200 single basidiospore isolates (SB) denominated as SB001 – SB200 (Figure 1(a) - (f)).

Morphological Characterization of Single Basidiospore Isolates

The 200 SBs were observed and characterized into three main groups which were scattered, rough and smooth group based on the mycelium appearance (Gupta *et al*., 2011). Under each main group, range of mycelium appearance and colony diameter growth after 7 days of post inoculation (CD-7dpi) were observed for each SB without replication.

Within each main group (scattered, rough and smooth), variations of mycelium appearance and CD-7dpi were observed. From each group, representative SBs which were considered different from each other in terms of mycelium appearance and CD-7dpi were selected and re-characterized with three replications (Figure 1(g)). There were six representative SBs selected from the smooth group, seven from scattered group and five from the rough group. The data on CD-7dpi obtained were analysed using One-way ANOVA using SPSS software. The representative SBs of the three main groups were then grouped into fast growing colony diameter (FGCD-7), medium fast growing colony diameter (MFGCD-7) and slow growing colony diameter (SGCD-7).

Hybridization Compatibility of Single Basidiospore Isolates

From the original 200 SB isolates, 10 FGCD-7 and 10 SGCD-7 were selected randomly for hybridization compatibility test. Two point inoculations of the selected SBs were performed with every possible pairing without repetition.

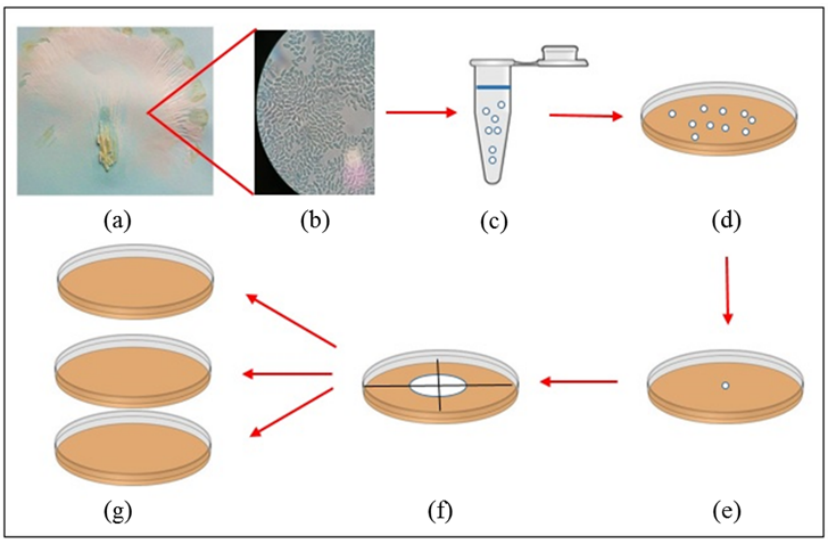

Figure 1. Source of spores and SB isolate culture, characterization and morphological observation, and replication of SB isolate of *P. sajor*-caju. (a) Spore print, (b) Spores observed under compound microscope, (c) Spore suspension, (d) 50 spores/200µL cultured on PDA media, (e) 200 spores were cultured individually on PDA media (SB isolates obtained were denoted as SB001-SB200), (f) 200 SB characterized into three main groups i.e, scattered, rough and smooth. Under each main group, range of morphological polymorphism, colour and CD-7dpi of the SB were observed, and (g) Observation with three replications to reconfirm the observed traits.

Each pair of SBs was placed 3 cm apart from each other in a Petri dish containing PDA. The plate was placed in room temperature until contact zone was observed between the pair of SBs. A strip of approximately 2 mm of mycelium was cut off from the contact zone, placed on new PDA culture and was allowed to grow for three to four days and examined under compound microscope (Nikon Eclipse E100) for the occurrence of clamp connections. The CD-7dpi of hybridized isolates was tested with three replications together with their respective parental single basidiospore isolates. The mating type of the selected FGCD-7 and SGCD-7 single basidiospore isolates was predicted using a tester strain that are compatible with each other. In this study, there were two attempts of hybridization. For the first attempt, SB003 (SGCD-7) and SB012 (FGCD-7) were selected as tester strains while for the second attempt, the tester strains were SB111 (FGCD-7) and SB125 (FGCD-7).

RESULTS AND DISCUSSION

Phenotypic Variations of Single Basidiospore Isolates

Mycelium appearance

From the 200 SBs observed, they are divided into three main groups based on colony morphology which are scattered, rough and smooth. The appearance of scattered, in this context refers to SB was defined as mixtures of morphologies found in random directions. Under the scattered group, different ranges of morphologies were recorded, from less scattered to very scattered (Figure 2). The less scattered ones such as SB029, SB133 and SB121 have their scattering mycelium ranged from floccose texture to interwoven hyphae towards the end of the plate (Figure 2(a), 2(b) and 2(c)). Those having medium scattering mycelium are ranged from woolly to snowy like appearances such as SB023 and SB007 (Figure 2(d) and 2(e)). For those having very scattered mycelium (SB106 and SB008), the scattering morphology ranged from dense snowy like appearances with large grains to mycelial tufts protruding from the groups of hyphae (Figure 2(f) and 2(g)).

Rough refers to SB without the presence of cottony structure that are either coarse, harsh, bumpy or wrinkled. For rough group, it was further categorised from less rough to very rough mycelium texture (Figure 3). Less rough mycelium texture ranged from lacunose texture to chalky texture such as SB138 and SB124 (Figure 3(a) and 3(b)). For rough mycelium texture, the mycelium shows chalky-crustose texture (SB081; Figure 3(c)). Those having very rough mycelium texture such as SB016 and SB113 showed morphology ranged from lacunose-granular to chalky-granular texture (Figure 3(d) and 3(e)).

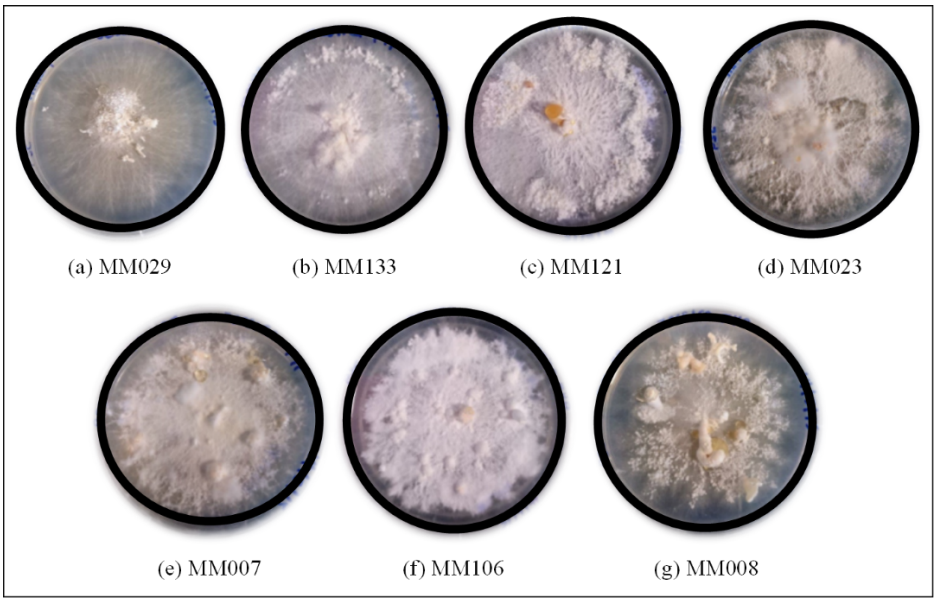

Figure 2. The range of scattered colony morphology of *P. sajor-caju*. (a) - (c) Less scattered, (d) - (e) Mediumly scattered, and (f) - (g) Very scattered.

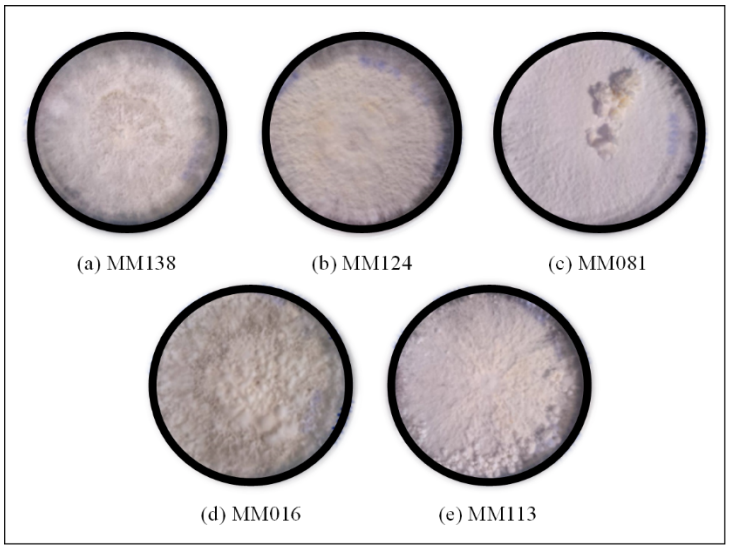

Figure 3. The range of rough colony morphology of *P. sajor-caju*. (a) - (b) Less rough, (c) Rough, and (d) - (e) Very rough.

Smooth in this context refers to SB with the presence of cottony texture with or without fine-grained structure. Under the smooth group, the mycelium morphology ranged from less smooth to very smooth (Figure 4). Less smooth mycelium texture ranged from cottony, woolly to lacunose texture such as SB137 and SB037 (Figure 4(a) and 4(b)). For smooth mycelium texture, the mycelium shows a cottony-zonate characteristics (SB047; Figure 4(c)). For those having very smooth mycelium texture like SB130, SB083 and SB005 ranged from downy to cottony (Figure 4(d), 4(e) and 4(f)).

Variations in mycelium appearance for SBs were also observed for *P. sajor caju* in India by Gupta *et al*. (2011). In addition, Gupta *et al*. (2011) observed colour variations ranged from dull white to yellow pigmented nut was not observed for the 200 SBs of this study.

Colony Diameter Growth

Besides the variations of mycelium appearance observed in each group, the SBs can also be categorised based on their CD-7dpi in the respective groups. In all scattered, rough and smooth groups, the SBs were grouped into three categories of CD-7dpi which were, slow growing CD-7dpi (SGCD-7), medium fast growing CD-7dpi (MFGCD-7) and fast growing CD-7dpi (FGCD-7) (Table 1). Based on One-way ANOVA, the SGCD-7 in the scattered group was

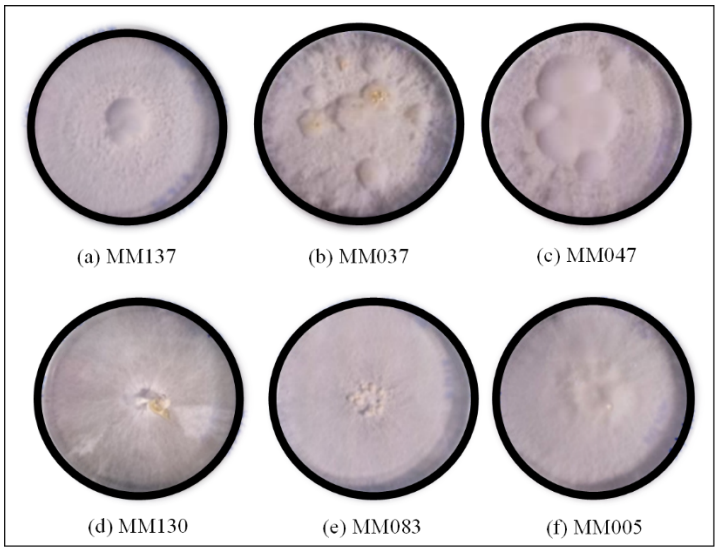

Figure 4. The range of smooth colony morphology of *P. sajor-caju*. (a) - (b) Less smooth, (c) Smooth, and (d) - (f) Very smooth.

Table 1. Categories of CD-7dpi in Scattered, Rough and Smooth groups of *P. sajor-caju*.

CD-7dpi Groups	SGCD-7 Range (cm)	MFGCD-7 Range (cm)	FGCD-7 Range (cm)
Scattered	4.3-5.6 (SB007, SB008, SB023, SB121)	7.1-7.2 (SB029, SB133)	8.0 (SB106)
Rough	4.1-4.3 (SB124, SB138)	5.7-6.2 (SB016, SB113)	8.0 (SB081)
Smooth	5.8-6.8 (SB005, SB037, SB138)	7.4-7.5 (SB083)	7.9-8.0 (SB047, SB137)

Note: All single basidiospore isolates in each category of SGCD-7, MFGCD-7 and FGCD-7 were significantly differently from single basidiospore isolates of different category based on One-way ANOVA.

approximately ranged from 4.3-5.6 cm, for MFGCD-7 was 7.1-7.2 cm and FGCD-7 was equal or more than 8 cm. From a total of seven selected SBs under scattered group, four SBs belongs to SGCD-7 category, two SBs in MFGCD-7 category, and one SB in FGCD-7 category. For the rough group, SGCD-7 was approximately ranged from 4.1-4.3 cm, for MFGCD-7 was 5.7-6.2 cm and FGCD-7 was equal or more than 8 cm. From a total of five selected SBs under rough group, two SBs falls under SGCD-7 category, two SBs under MFGCD-7 and one SB under FGCD-7 category. In the smooth group, SGCD-7 was approximately ranged from 5.8-6.8 cm, for MFGCD-7 was 7.4-7.5 cm and FGCD-7 was 7.9-8.0 cm. Out of six selected SBs under smooth group, three SBs were SGCD-7, one SB was MFGCD-7 and two SBs FGCD-7 categories respectively. Gupta *et al*. (2011) also observed variations in the growing rate for *P. sajor caju* in India. The presence of morphological variations provides an opportunity for strain improvement.

Hybridization Compatibility

In this study, colony diameter growth is considered in selection for generating hybridized isolate. There were ten FGCD-7 (1 scattered SB, 3 rough SBs and 6 smooth SBs) and ten SGCD-7 (4 scattered SBs, 3 rough SBs and another 3 smooth SBs) randomly selected from the 200 SBs for hybridization compatibility test. Two SBs were considered as compatible when clamp connection was observed which indicates heterokaryotic formation as described for *P. ostreatus* (Gharehaghaji *et al*., 2007).

The mating system of *P. sajor caju* is tetrapolar (Gupta *et al*., 2011) as observed in other *Pleurotus* species (Esser & Blaich 1994; Gharehaghaji *et al*., 2007). Mating type loci A and B are the two genetic loci that determine the mating type in *Pleurotus* species. When a cell with mating type of A_xB_x mate with another cell having A_yB_y mating type, hybridized isolate would formed having the combination of distinct proteins in one cytoplasm (Kothe, 2001). SBs which are compatible with each other were selected as tester strains to identify the mating types of other SB.

SB003 and SB012 were selected as tested strains designated as A_xB_x and A_yB_y, respectively. Out of the ten randomly selected SBs in the first hybridization attempt (5 FGCD-7 and 5 SGCD-7 inclusive SB003 and SB012), four SBs were compatible with SB003 but not with SB012. They were of the mating type A_yB_y opposite to that of SB003 (A_xB_x). Two SBs were compatible with SB012 but not with SB003. Their mating type was A_xB_x. There were four SBs failed to show any compatibility with both the tester strains (Table 2). SB111 and SB125 were selected as tested strains and designated as the mating types A_mB_m and A_nB_n, respectively. Out of ten randomly selected SBs (5 FGCD-7 and 5 SGCD-7 inclusive SB111 and SB125), four SBs were compatible with SB111 and were given the mating type A_nB_n. Two SBs were compatible with SB125 and were given the mating type A_mB_m. The other four SBs were not compatible with both the tester strains (Table 2). The SBs which did not show compatibility with the respective tester strain are probably due repulsion through self and nonself recognition (Kronstad & Staben, 1997; Micali & Smith, 2005). Demarcation line was observed which keep away genetically different mycelia (Figure 5).

Table 2. SBs *Vs* tester strains for deduction of mating type for each SB of *P. sajor-caju*. 'O' indicate compatibility while 'X' indicate incompatibility.

First hybidisation attempt	Categories of CD-7dpi	Tester strain SB003 (A_xB_x)	Tester strain SB012 (A_yB_y)
SB015	SGCD-7	O	X
SB047	SGCD-7	O	X
SB003	SGCD-7	X	O
SB034	SGCD-7	X	X
SB008	SGCD-7	X	X
SB012	FGCD-7	O	X
SB023	FGCD-7	O	X
SB014	FGCD-7	X	O
SB036	FGCD-7	X	X
SB046	FGCD-7	X	X
Second hybridisation attempt		Tester strain SB111 (A_mB_m)	Tester strain SB125 (A_nB_n)
SB069	SGCD-7	O	X
SB091	SGCD-7	O	X
SB094	SGCD-7	O	X
SB086	SGCD-7	X	X
SB130	SGCD-7	X	X
SB125	FGCD-7	O	X
SB111	FGCD-7	X	O
SB112	FGCD-7	X	O
SB055	FGCD-7	X	X
SB127	FGCD-7	X	X

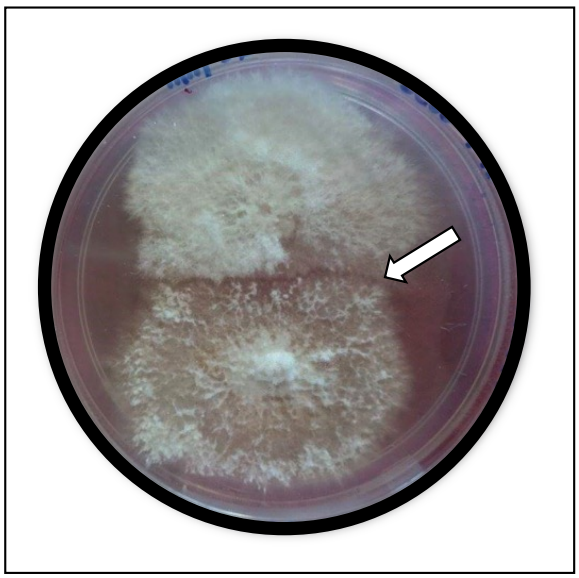

Figure 5. The arrow shows the demarcation line between two incompatible SBs of *P. sajor-caju.*

In total, there were 16 hybridized isolates obtained. Out of the 16 hybridized isolates, five were from hybridizing SBs within FGCD-7 category, four were from hybridizing SBs within SGCD-7, and seven between SBs from FGCD-7 and SGCD-7 (Table 3). Based on the predicted mating type, all the 16 hybridized isolates obtained are indeed following the predicted compatibility. Only five hybridizations which were expected to be compatible did not result in hybridized isolate. The incompatibility may be due to the lab conditions which did not allow compatible mating (Ikeda *et al.,* 2002) i.e., the culturing environment is not suitable for isolate or hybridization condition.

Table 3. List of compatible pairings for each hybridization of selected SBs and their average CD-7 for both parental strains and hybridized isolates of *P. sajor-caju*.

Hybridisation	Mating type	Parental Strains Average CD-7 (cm)	Parental Strains Mycelium Appearance	Hybridized isolate Average CD-7 (cm)	Hybridized isolate Mycelium Appearance
Hybridisation within FGCD-7 SBs	SB014 (A_xB_x) SB012 (A_yB_y)	SB014 = 4.3 SB012 = 5.8	Smooth Smooth	8.0**	Smooth
	SB014 (A_xB_x) SB036 (A_yB_y)	SB014 = 4.3 SB036 = 5.6	Smooth Rough	7.2*	Rough
	SB046 (A_xB_x) SB023 (A_yB_y)	SB046 = 4.3 SB023 = 6.3	Rough Scattered	7.5*	Scattered
	SB111 (A_mB_m) SB125 (A_nB_n)	SB111 = 6.5 SB125 = 6.6	Smooth Smooth	8.0	Smooth
	SB112 (A_mB_m) SB125 (A_nB_n)	SB112 = 6.7 SB125 = 6.6	Smooth Smooth	8.0	Smooth
	Average	5.7		7.7	
Hybridisation within SGCD-7 SBs	SB086 (A_mB_m) SB091 (A_nB_n)	SB086 = 4.0 SB091= 3.9	Scattered Scattered	6.0**	Scattered
	SB003 (A_xB_x) SB015 (A_yB_y)	SB003 = 2.3 SB015 = 3.7	Smooth Smooth	5.6**	Smooth
	SB069 (A_nB_n) SB086 (A_mB_m)	SB069 = 3.2 SB086 = 3.9	Scattered Scattered	5.8*	Scattered
	SB003 (A_xB_x) SB047 (A_yB_y)	SB003 = 2.0 SB047 = 2.5	Smooth Rough	3.9	Rough
	Average	3.2		5.3	
Hybridisation between FGCD-7 and SGCD-7 SBs	SB012 (A_yB_y) SB003 (A_xB_x)	SB012 = 6.1 SB003 = 2.2	Smooth Smooth	7.8**	Smooth
	SB023 (A_yB_y) SB003 (A_xB_x)	SB023 = 6.3 SB003 = 2.2	Scattered Smooth	7.6**	Scattered
	SB014 (A_xB_x) SB015 (A_yB_y)	SB014 = 4.1 SB015 = 3.8	Smooth Smooth	7.2**	Smooth
	SB111 (A_mB_m) SB069 (A_nB_n)	SB111 = 6.5 SB069 = 3.1	Smooth Scattered	7.8*	Smooth
	SB127 (A_mB_m) SB069 (A_nB_n)	SB127 = 6.8 SB069 = 3.1	Smooth Scattered	7.7*	Smooth
	SB111 (A_mB_m) SB091 (A_nB_n)	SB111 = 6.7 SB091 = 4.2	Smooth Scattered	7.9*	Smooth
	SB111 (A_mB_m) SB094 (A_nB_n)	SB111 = 7.0 SB094 = 5.2	Smooth Scattered	7.8*	Smooth
	Average	4.8		7.7	

*Hybridized isolate was significantly different from one of its parents at $\alpha = 0.05$.
**Hybridized isolate was significantly different from both its parents at $\alpha = 0.05$.

For all the compatible hybridization, single colony was obtained from small piece of mycelium in the regions of interaction. The mycelium appearance of the hybridized isolates were similar to the parents (Figure 6). Each of the parental SB and their hybridized isolates were compared based on CD-7dpi. The growth of nearly all the hybridized isolates from FGCD-7 pairings, SGCD-7 pairings, and between FGCD-7 and SGCD-7 pairings, was significantly faster than at least one of its parents (Table 3). Such observation is also true for *P. ostreatus* (Gharehaghaji *et al*. (2007). The hybridized isolates will be characterized further for spawning rate and yield.

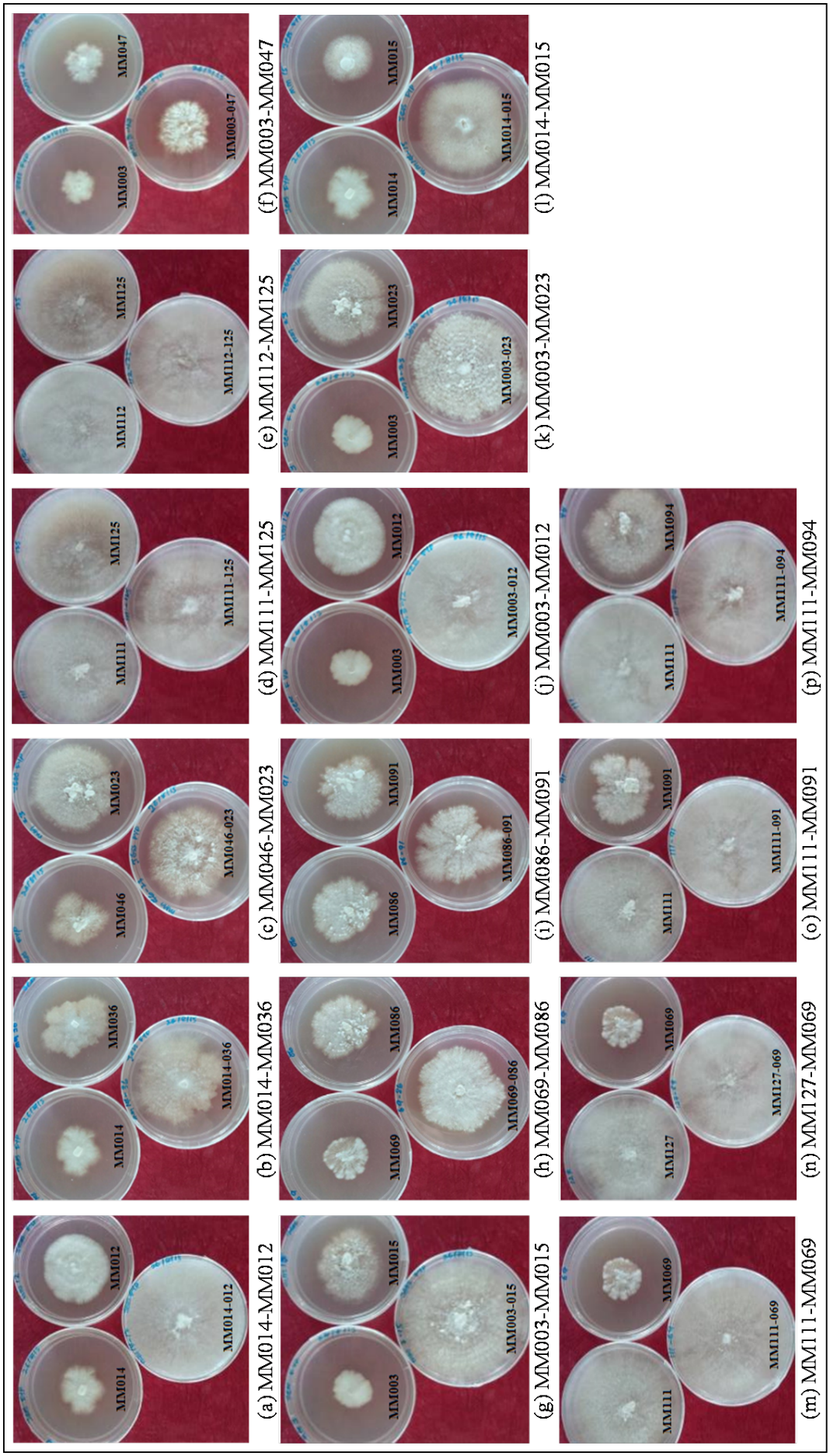

Figure 6. Parental SB isolates and hybridized isolates of *P. sajor-caju*. (a) – (e) Pairings within FGCD-7, (f) – (i) Pairings within SGCD-7, and (j) – (p) Pairings between FGCD-7 and SGCD-7.

CONCLUSION

In conclusion, this study has documented the variations observed on the SBs, isolated from a commercialized strain of *P. sajor caju* cultivated in Kuching, Sarawak. A total of 16 hybridized isolates are obtained from hybridizing a selection of SBs which will be characterized further for their potential as breeding lines.

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