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MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF FUNGI AND BACTERIA ASSOCIATED TO COMMON SPEAR ROT DISEASE IN MALAYSIA

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ABSTRACT

*Common spear rot (CSR) also known as crown disease (CD), is a disease known to affect young immature oil palm (*Elaeis guineensis *Jacq.) in the field. However, there is limited information on CSR disease incidences reported in Malaysia. Hence, this study was aimed to identify possible pathogens causing CSR disease on oil palm in Malaysia. Palm showing severe lesions and rotting of unopened spear leaves was identified, and the internal tissues from healthy and infected bole sections were sampled. Five fungal and 12 bacterial isolates were recovered from the infected tissues, while three fungal and two bacterial isolates were isolated from healthy tissue. Macroscopic identification of these cultures was conducted by observing the bacterial and fungal isolates grown in nutrient agar (NA) and potato dextrose agar (PDA), respectively. Molecular identification was carried out using internal transcribed spacer (ITS), translation elongation factor (TEF 1-α) and 16S primers through polymerase chain reaction (PCR). The identity of each isolate was determined using the BLASTN program through non-redundant database nucleotide collection. The sequence analysis showed most of the fungal isolates isolated were identical to* Fusarium *genus with 96.35% to 100.00% similarity when compared to sequences deposited in the GenBank. The species* F. solani *was one of the most frequently recovered fungal isolates from the infected tissues. Meanwhile, one* Erwinia *sp., nine* Klebsiella *sp., three* Dickeya *sp. and one* Enterococcus *sp. were identified from the bacterial collection. Phylogenetic analysis revealed that all isolates of* F. solani *from the diseased palm clustered together with* F. solani *belonging to other hosts, validating the identity of the isolates. Apart from that,* Klebsiella *sp. was also isolated and could also be responsible for causing CSR but requires further validation through Koch's postulate assessment. Nevertheless, this is the first study reporting the isolation of* Klebsiella *sp. in diseased CSR oil palm.*

Keywords: bacteria, common spear rot, fungi, oil palm, phylogenetic tree.

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INTRODUCTION

Common spear rot (CSR) also known as crown disease (CD) is commonly found affecting oil palm during the juvenile years especially in the first two years after field transplanting. It has been reported that CSR/CD occurs in all regions where oil palms are grown (Corley and Tinker, 2008; Duff, 1963; Turner, 1981). The disease was first reported in North Sumatera in the 1920s (Turner, 1981). There have been occasional reports of isolated cases of CSR on oil palm in Malaysia, where incidences rarely exceeded 1%. However, the disease has attracted considerable amount of attention in Malaysia due to the lack of information on potential pathogen identified as the causal agent.

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The term CSR was used by Chinchilla-López (2008) to describe the disease with or without the typical characteristics of rachis bending and rotting that does not progress towards meristem. During the development of the disease, the young leaves become shorter and distorted, followed by rachis bending characteristics manifested due to the reduction in lignification process in the parenchyma's tissues and this appears especially when the disease was under favourable condition (Hartley, 1988) and the emergence of rotting and drying in some sections of the spears. Another symptom describing CSR is that the tissues at the tips of the youngest leaves appear rotting or known as wither tip (Turner, 1981). Extensive rotting may affect the meristem which leads to the death of the palm, otherwise it is possible to recover provided the rotting tissue does not progress towards meristem. Additionally, the incidences of CSR do not manifest immediately but occurs between 12 to 18 months after transplanting from the nursery in a dry season followed by rainy period after replanting (Suwandi *et al.*, 2012).

Several isolates were recovered from diseased leaf tissues of oil palms in Indonesia such as *Fusarium incarnatum*, *Fusarium sacchari* and *Ceratocystis paradoxa* (Suwandi *et al.*, 2012). Pathogenicity test revealed that *C. paradoxa* caused the rotting symptoms and upon inoculation on the leaves, the lesion enlarged rapidly. The lesion also turned from brownish to blackish which is a typical symptom of CSR in the field (Suwandi *et al.*, 2012). Considering the fact that *C. paradoxa* is a common fungus recovered from various diseases of oil palm, its association with the CSR lesion is still not well established. Another study reported that high dosages of nitrogen and poor drainage condition also promoted the oil palm's susceptibility and led to CSR (Alvarado-Vega *et al.*, 1996). This was also demonstrated in the study by Suwandi *et al.* (2012) whereby seedlings were subjected to drainage for nine days before inoculation, followed by no watering for six days before being flooded with freshly prepared fertiliser solution which resulted in the increased seedlings' susceptibility to CSR infection. Other predisposing factors that were also identified were the soil conditions including soil compaction and water saturation, imbalance of nutrients in particular nitrogen, which may contribute to the incidences of spear rot and rachis bending characteristics (Turner, 1981; Alvarado-Vega *et al.*, 1996; Breure and Soebagjo, 1991; Chinchilla-López *et al.*, 1997). However, due to the scarcity of the studies conducted, there is inadequate findings to conclude these observations. Moreover, there is also lack of studies conducted on CSR affected palms in Malaysia to verify the results reported in the previous studies. Therefore, this study was initiated with the primary objective to investigate on the possible pathogens that could be associated for the occurrence of CSR in the younger palms in Malaysia.

MATERIALS AND METHODS

Sampling of Diseased Tissue

An infected 3 year-old palm with rotting observed at the tips of younger leaves (*Figure 1*) was removed at the basal area in order to examine the degree of infection. For sampling purpose, the bole and roots of the palm were removed. Selected roots were detached while the bole was cross sectioned horizontally into smaller sections and brought into the laboratory for further analysis.

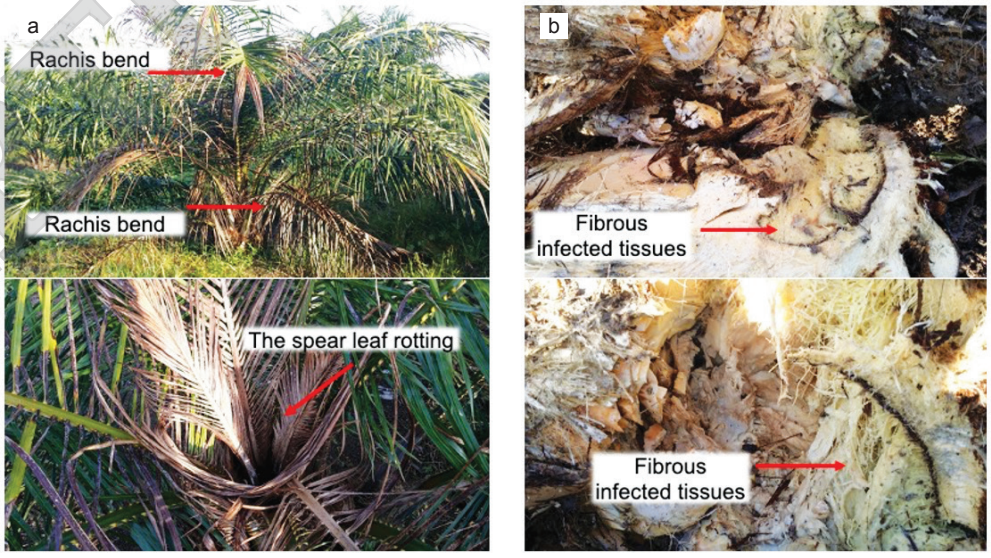


Figure 1. Symptoms of common spear rot (CSR) of oil palm. a) Emergence of spear rot on 3 year-oil palm, b) cross-section of oil palm basal area, revealing the fibrous infected tissues at the inner part.

Isolation of Fungal and Bacterial Strains

Isolation from infected bole. Large portion of infected oil palm bole was carefully removed and cut into smaller pieces for ease of handling during the isolation process. The bole pieces were then cut into even smaller pieces aseptically in a biosafety cabinet. The marginal area of actively developing lesions from diseased tissue were excised and plated onto quarter strength potato dextrose agar (¼ PDA) (Difco™, USA) for the isolation of fungal strains. Healthy/uninfected regions were identified on the infected bole were also carefully excised and plated onto the same media. Plates were incubated for three to five days at 28°C to allow for fungal growth. Isolated colonies were purified and maintained on ¼ PDA. Pure cultures of these colonies were maintained on ¼ PDA slants as well as in sterile distilled water in universal bottles and stored in incubator at 28°C. Meanwhile, nutrient agar (NA) (Oxoid, United Kingdom) was used for the isolation of bacterial colonies. The NA plates were incubated for two to three days at 37°C. The emerging bacterial colonies were purified and maintained on NA plates. The obtained pure strains were stored in sterile distilled water and stored at 4°C.

Molecular Identification of Pure Cultures

The deoxyribonucleic acid (DNA) of fungal and bacterial cultures were extracted using microLYSIS-Plus (Microzone, United Kingdom) according to manufacturer's instructions. Samples were amplified by polymerase chain reaction (PCR) using TW81: 5′-GTTTCCGTAGGTGAACCTGC-3′ and AB28: 5′-ATATGCTTAAGTTCAGCGGGT-3′ primer pairs to amplify ITS1 and ITS2 regions for fungal strains (Howlett *et al*., 1992) and using TEF1 5′-ATGGGTAAGGA(A/G)GACAAGAC-3′ and TEF2 5′-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3′ to amplify the TEF 1-α gene region (O'Donnell *et al*., 1998) and 16S-F: 5′-GAGTTTGATCCTGGCTCAG-3′ and 16S-R: 5′-CGGCTACCTTGTTACGACTT-3′ for bacterial strains. The PCR reaction mixture containing 1 × Taq DNA polymerase buffer (Invitrogen, Brazil), 0.5 mm dNTPs (Biolab, USA), 1.25 mm of magnesium chloride ($MgCl_2$) (Invitrogen, Brazil), 0.5 mm of each primer, 2 µl of DNA template, 1 U/µl of recombinant Taq DNA polymerase (Invitrogen, Brazil) and sterile distilled water was added to make up a final volume of 50 µl. PCR was performed in a Vapoprotect™ Mastercycler Pro S (Eppendorf, Germany) with initial denaturation condition at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 60 s; and a final extension at 72°C for 10 min. The PCR products were run on 1.5% gel electrophoresis at 80 V for 30 min. The amplified region was excised, extracted using QIAquick® Gel Extraction Kit (QIAGEN) and sent for sequencing.

Phylogenetic Analysis

The raw sequence was cleaned using Applied Biosystem Sequence Scanner Software by viewing the base quality value (QV) score of >20. ITS sequences of each individual isolate were compared to those in non-redundant protein database in GenBank using BLASTN program available at the NCBI website (http://www.ncbi.nlm.nih.gov) with cut-off E-value ≤ $1e^{-5}$. The sequence data of internal transcribed spacer (ITS), translation elongation factor 1-α (TEF 1-α) and 16S were aligned separately with other sequence data retrieved from GenBank database to determine the genetic relationship between the nucleotides as listed in *Tables 1-3*, respectively. Phylogenetic tree was constructed using MEGA 6.06 (Tamura *et al*., 2013) and Neighbour-Joining statistical method with 1000 bootstrap replications via estimation of pairwise distances.

RESULTS AND DISCUSSION

Isolation of Fungi and Bacteria

Five fungal isolates were recovered from infected tissues (*Table 4*). The identity of the representative isolates using ITS were *F. solani* and *Fusarium* sp. *Fusarium solani* (three isolates) was most frequently isolated, whereas the *Fusarium* sp. was only isolated once. Additionally, *Fusarium* sp. (two isolates) and *Fusarium verticillioides* were found in the healthy tissue. The *Fusarium* spp. were further identified using TEF-1α (*Table 4*). Among the non-*Fusarium* species, *Cryptococcus* sp. was also retrieved from infected tissues. Meanwhile, 12 bacterial isolates were recovered from infected tissues including *Klebsiella pneumoniae* (two isolates); *Klebsiella variicola* (three isolates); *Klebsiella* sp. (three isolates); *Dickeya zeae* (two isolates); *Erwinia chrysanthemi* and *Enterococcus gallinarum* were isolated once (*Table 5*). The culture plates of the isolates recovered from healthy tissues are shown in *Figure 2* while the plates of isolates from infected tissues are shown in *Figure 3*. The morphology of each culture plate was described in the respective figures.

DNA Sequencing and Phylogenetic Analysis

A PCR based method is a popular method in microbial study to understand the evolutionary relationship of isolated cultures. This approach uses DNA sequences referred to as primers which bind at specific DNA sequences and amplify the

TABLE 1. LIST OF INTERNAL TRANSCRIBED SPACER (ITS) SEQUENCES DATA OF FUNGAL SPECIES RETRIEVED FROM GENBANK

No.	GenBank accession	Species name	Strain ID	Size of rRNA ITS region (bp)	Origin	Host plant/source
1.	MK174969.1	*Fusarium verticillioides*	171779	540	Mexico	-
2.	KY495190.1	*F. verticillioides*	CGARSF	1 149	Nigeria	Rhizosphere soil
3.	MG561965.1	*Fusarium* sp.	Endophytic fungi	526	China	*Bupleurum chinense* DC.
4.	EF680757.1	*Fusarium* sp.	TN2-86032	549	India	*Saccharum officinarum* (sugarcane)
5.	GU595038.1	*F. solani*	H4470	566	China	*Rhizophoraceae* sp. (mangrove)
6.	HQ248197.1	*F. solani*	PCO.30	567	Colombia	*Elaeis guineensis* (oil palm)
7.	KF679356.1	*F. solani*	1HBF 2353	573	India	*Aquilaria agallocha* (agarwood)
8.	EU871517.1	*Cryptococcus magnus*	S22814	651	India	*Filobasidium magnum*
9.	JQ425367.1	*C. magnus*	AUMC7772	623	Egypt	*Filobasidium magnum*
10.	MH401206.1	*Phytophtora infestant*	13-A2	883	United Kingdom	*Solanum tuberosum*
11.	FJ801316.1	*P. palmivora*	WPC10213A717	786	United State	-
12.	KF939052.1	*Cerotocystis paradoxa*	BX3	497	China	*Butia capitata*

TABLE 2. LIST OF TRANSLATION ELONGATION FACTOR 1-α (TEF 1-α) SEQUENCES DATA OF FUNGAL SPECIES RETRIEVED FROM GENBANK

No.	GenBank accession	Species name	Strain ID	Size of rRNA ITS region (bp)	Origin	Host plant/source
1.	MT119148.1	*Fusarium solani*	FSCS8	789	India	-
2.	HM770727.1	*F. solani*	SB 21	701	Indonesia	Leaflet, oil palm
3.	MN193859.1	*F. brevicatenulatum*	NRRL 25447	1 768	Guyana	*Xyris spp.*
4.	JF270183.1	*F. incarnatum*	Spt 017	702	USA	Sorghum
5.	JF270304.1	*F. incarnatum*	Spt 142	697	USA	Sorghum
6.	HM569630.2	*Thielaviopsis paradoxa*	C1481	1 492	USA	Oak

TABLE 3. LIST OF 16S SEQUENCES OF BACTERIAL SPECIES RETRIEVED FROM GENBANK

No.	GenBank accession	Species name	Strain ID	Size of rRNA ITS region (bp)	Origin	Host plant/source
1.	GU811708.1	*Erwinia crysanthemi*	PY-3	1 491	China	Banana
2.	GU362079.1	*E. crysanthemi*	PD2098	1 499	India	Aloe vera
3.	KJ8337551	*E. crysanthemi*	BM01	1 454	China	Banana
4.	JQ398851.1	*Klebsiella* sp.	VITC1	1 444	India	Cinnamon fed rat
5.	MK905714.1	*Klebsiella* sp.	RIB-SCM15	1 387	Nepal	Banana
6.	MN725742.1	*K. variicola*	SA002	1 411	Colombia	*Aedes aegypti* (L.)
7.	MN725749.1	*K. variicola*	SA006	1 415	Colombia	*Aedes aegypti* (L.)
8.	KJ438947.1	*Dickeye. zeae*	Dz-7	1 414	Mexico	Maize
9.	KJ438946.1	*D. zeae*	Dz-7	1 414	Mexico	Maize
10.	MH9030397.1	*Klebsiella pneumonia*	TZT-18-63	1 441	China	Banana
11.	MN749602.1	*K. pneumonia*	GMH480	1 071	Iraq	Water sample
12.	MK902672.1	*K. pneumonia*	SK1	1 406	India	-
13.	MT573855.1	*Enterococcus gallinarum*	1465	1 385	China	-
14.	MT158593	*E. gallinarum*	G116	1 411	China	-

TABLE 4. FUNGAL ISOLATES RECOVERED FROM INFECTED AND HEALTHY TISSUES OF THE PALM

Isolates ID	Annotation using ITS primer	Percent identity (%)	GenBank accession no.	Annotation using TEF 1-α primer	Percent identity (%)	GenBank accession no.
Fungal Isolates						
SPEAR ROT: INFECTED TISSUES (ACTIVELY GROWING LESIONS)						
SR1fi	*Fusarium solani* (KU377470.1)	100	MW 314726	*F. solani* (KX497028.1)	99.86	Submitted and awaiting assignment of accession number
SR2fi	*F. solani* (KU377470.1)	100	MW 314727	*F. solani* (KX497028.1)	100	saa
SR3fi	*Cryptococcus* sp. (HQ426594.1)	100	MW 314728	*F. solani* (MT119148.1)	100	saa
SR7fi	*F. solani* (KU377470.1)	100	MW 314730	*F. solani* (MT119148.1)	99.73	saa
SR8fi	*Fusarium* sp. (MT636465.1)	99.56	MW 314731	*F. brevicatenulatum* (MT011005.1)	96.35	saa
SPEAR ROT: HEALTHY TISSUES						
SR1fh	*Fusarium* sp. (MT636465.1)	100	MW 314732	Not amplified	-	saa
SR3fh	*Fusarium* sp. (MT636465.1)	100	MW 314733	*F. incarnatum* (JF 270296.1)	99.19	saa
SR4fh	*F. verticillioides* (MT035918.1)	99.4	MW 314734	Not amplified	-	saa

Note: saa - same as above.

TABLE 5. BACTERIAL ISOLATES RECOVERED FROM INFECTED AND HEALTHY TISSUES OF THE PALM

Isolates ID	Accession no.	Annotation	Percent identity (%)	GenBank accession no.
Bacterial Isolates				
SPEAR ROT: INFECTED TISSUES (ACTIVELY GROWING LESIONS)				
SR1bi	GU811708.1	*Erwinia chrysanthemi*	97.58	MW334951
SR2bi	KJ42495.1	*Klebsiella pneumoniae*	100	MW334952
SR3bi	MN082124.1	*K. variicola*	100	MW334953
SR4bi	KR399998.1	*Klebsiella* sp.	100	MW334954
SR5bi	CP025799.1	*Dickeya zeae*	100	MW334955
SR6bi	MN082124.1	*K. variicola*	100	MW334956
SR7bi	KR399998.1	*Klebsiella* sp.	100	MW334957
SR8bi	MT597704.1	*Enterococcus gallinarum*	100	MW334958
SR9bi	MN082124.1	*K. variicola*	100	MW334959
SR10bi	CP025799.1	*D. zeae*	99.88	MW334960
SR11bi	MH930397.1	*K. pneumoniae*	99.82	MW3349561
SR12bi	KR399998.1	*Klebsiella* sp.	100	MW3349562
SPEAR ROT: HEALTHY AREA				
SR1bh	KR399998.1	*Klebsiella* sp.	100	MW334963
SR2bh	CP025799.1	*D. zeae*	100	MW334964

targeted sequences (Mitchell and Zuccaro, 2006). The primers used are conserved molecular markers such as 18S ribosomal RNA (rRNA), 28S rRNA, ITS region (Innis *et al.*, 2012) and the TEF-1α gene, which encodes an essential part of the protein translation machinery. ITS region is one of the most commonly used markers in the phylogenetic study of most fungi, however, many *Fusarium* possess non-orthologous copies of the ITS, which can lead to incorrect phylogenetic inferences (O'Donnell

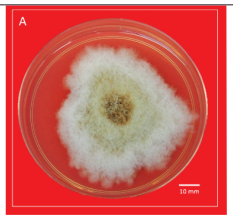
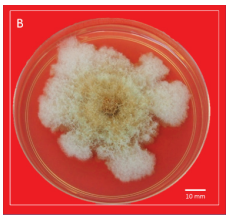
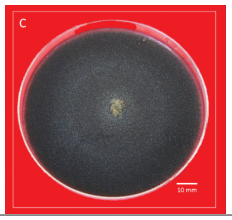
Isolates ID	Identity based on TEF 1-α	Cultures plates	Mycelium characteristics
SR1fh	unknown		Creamy with fluffy and cottony growth of mycelia. The mycelia appeared light yellowish at the centre
SR3fh	*Fusarium incarnatum*		Uneven creamy with fluffy and cottony growth of mycelia. The mycelia appeared yellowish at the centre
SR4fh	unknown		Blackish with flat and dense growth of mycelia

Figure 2. Morphology of isolates recovered from healthy tissues. a) SR1fh (unknown), b) SR3fh (Fusarium incarnatum)*, and c) SR4fh (unknown).*

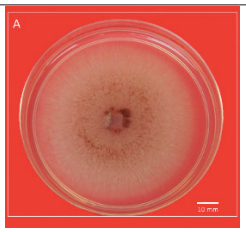
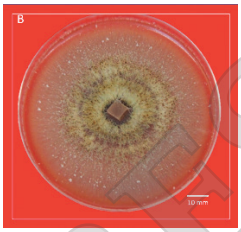
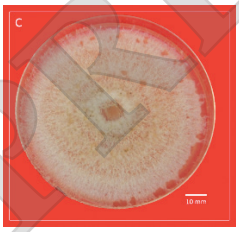
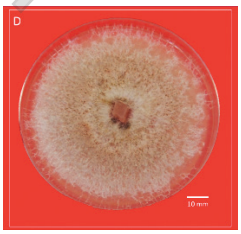
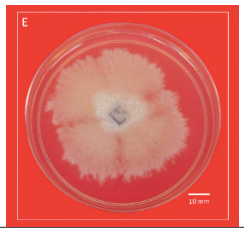
Isolates ID	Identity based on TEF 1-α	Cultures plates	Mycelium characteristics
SR1fi	*Fusarium solani*		Creamy off white with flat and fine growth of mycelia
SR2fi	*F. solani*		Creamy grey with flat but dense growth of mycelia. Mature mycelia will be observed with yellowish dense mycelia and sporulation
SR3fi	*F. solani*		Creamy, cottony with dense growth of mycelia
SR7fi	*F. solani*		Creamy, cottony with dense growth of mycelia
SR8fi	*F. brevicatenulatum*		Creamy, clumpy and thin layer growth of mycelia

Figure 3. Morphology of fungal isolates recovered from infected tissues. a) SR1fi (Fusarium solani)*, b) SR2fi* (F. solani)*, c) SR3fi* (F. solani)*, d) SR7fi* (F. solani)*, and e) SR8fi* (F. brevicatenulatum)*.*

et al., 1997). Therefore, the TEF-1α gene was also used in this study, whereby it has high phylogenetic utility due to its highly informative at the species level in *Fusarium*. TEF-1α is a non-orthologous copy of the gene that have not been detected in the genus and it is a universal primer designed to detect the genus (O'Donnell *et al.*, 1998). Similar to 16S rRNA, the region is a portion of the 30S small subunit of a prokaryotic ribosomal that binds to the Shine-Dalgarno sequence and is used in the reconstruction of phylogenetic trees due to the slow evolution of this gene region (Schuster *et al.*, 2002).

A single band approximately 600-700 bp was amplified using ITS primer while ~716 bp was amplified using TEF-1α primer. The similarity search of BLASTN against NCBI database is shown in *Table 4*. From the BLASTN annotation, the retrieved sequences matched to that of *Cryptococcus* sp. upon using ITS primer SR3fi but matched *F. solani* when TEF-1α primer sequencing results were used. This is because identification solely based on ITS can lead to misleading identification as two non-orthologous sequences can be isolated from a single isolate. Meanwhile, isolates SR8fi and SR3fh that annotated as *Fusarium* sp. upon using ITS primer was later shared similarity with *F. brevicatenulatum* and *F. incarnatum* respectively which proved the latter primers are highly informative at species level in *Fusarium*. *Fusarium* spp. has been identified before in previous studies as endophytes in oil palm (Rusli *et al.*, 2016) and their presence in the infected tissues is either as secondary opportunistic pathogen due to the compromised status of the affected CSR palms. Isolates SR1fh and SR3fh were not amplified when using TEF-1α primer and this could be due to the non-specific primer and may require other species - specific primers for further validation.

All the sequences were submitted to GenBank with accession number MW 314726 - MW 314734 for ITS while TEF sequences were submitted and awaiting accession number assignment. The phylogenetic trees were constructed with 1000 bootstrap and Neighbour-Joining method was found to group both *C. magnum* and *Phytophtora*

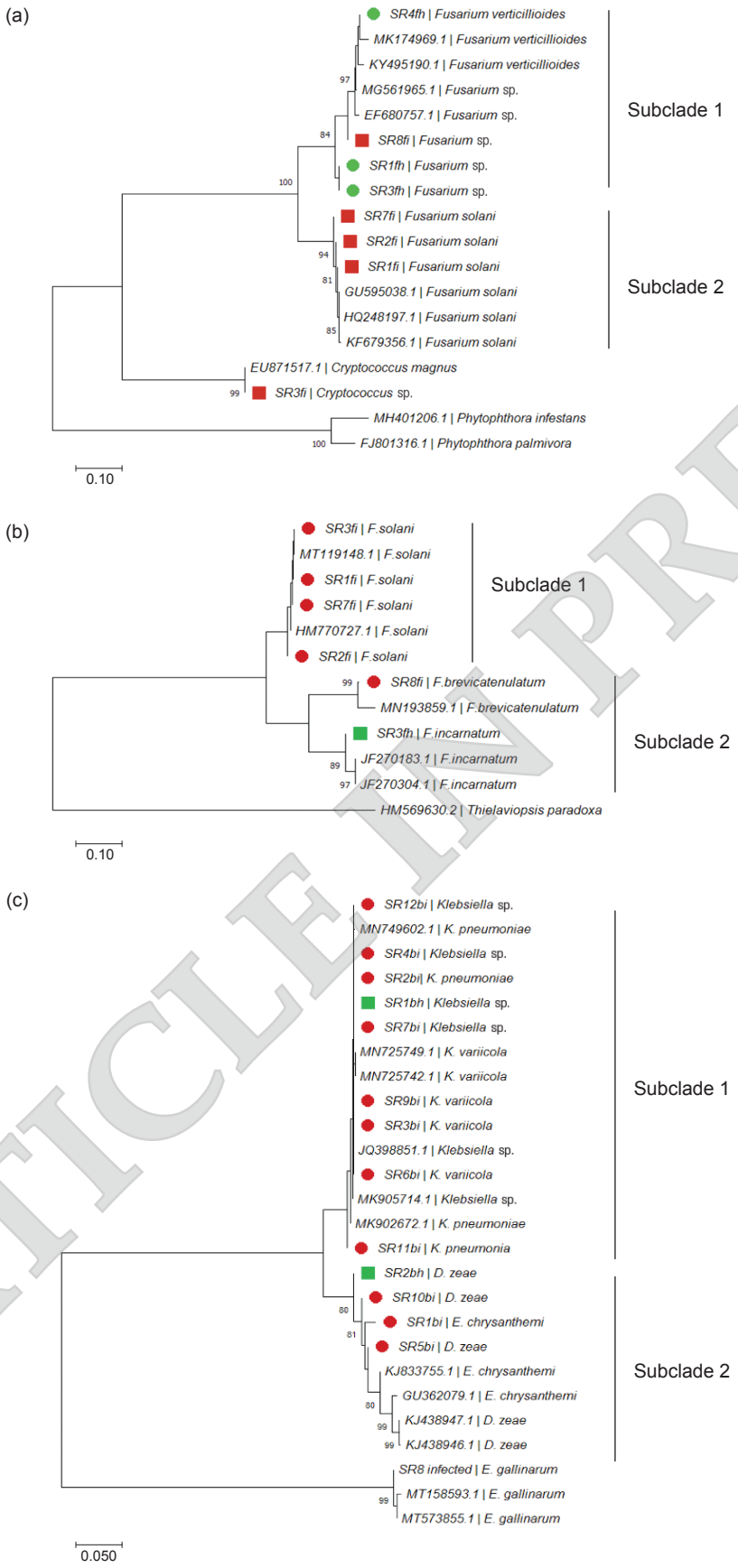


Figure 4. The evolutionary relationship of fungal isolates recovered from common spear rot (CSR) tissues were conducted in MEGA X. The phylogenetic tree was inferred using the Neighbour-Joining method from (a) ITS region, (b) TEF-1α region, and (c) 16S region obtained from NCBI, respectively. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method. The green circle represent isolates from healthy tissue and the red square represent isolates from infected tissues.

species as an outgroup clade respectively from the *Fusarium* species with ITS (*Figure 4a*) while *Thielaviopsis paradoxa* was noted as an outgroup clade for TEF (*Figure 4b*). Both phylogenetic trees of *Fusarium* were divided into two subclades supported by 100% bootstrap showing that most of the fungal isolates isolated from infected tissues falling under the *F. solani* subclades suggesting that the particular species may also be the causal agent for CSR. *Fusarium solani* causes crown and root rot on several crops such as strawberry (Pastrana *et al.*, 2014), soybean (Abood *et al.*, 2020) and potato (Falert and Akarapisan, 2019) which also appropriately elucidates the presence of the species in the infected tissues in this study. This finding is also in agreement with Turner (1981) whereby *F. solani* was reported as the predominant species isolated from CSR infected tissues. Previous studies have reported that inoculation of *Fusarium* sp. on oil palm leaves has been proven to cause wither tips symptoms of CSR affected palms (Turner and Bull, 1967).

As for bacterial isolates, approximately 1500 bp PCR product was amplified using 16S primer pairs and the sequences was submitted to GenBank with accession number of MW334951-MW334964 (*Table 5*). Phylogenetic tree showed that most of the strains recovered from infected tissue were grouped in the subclade of *Klebsiella* species (*Figure 4c*). Meanwhile, three isolates fall into the group of *Erwinia* species. *Klebsiella pneumoniae* is one of the isolates identified from infected tissues and known as an endophyte bacterium that can promote plant growth or control plant disease (Iniguez *et al.*, 2004) but it has been reported to also cause disease in maize (Huang *et al.*, 2016). The *K. pneumoniae* strain Borkar was reported to affect the root systems of *Solanaceous* plants, pomegranate or known as root bark necrosis disease. The bacterium inhibits the plant respiration mechanism creating anaerobic condition to the roots and leads to the emission of organic compounds, alcohol which results in a foul smell in the root and wilting in plants (Ajayasree and Borkar, 2018). Besides this, *K. variicola* was also reported to cause soft rot in banana (Fan *et al.*, 2016) and carrot (Chandrashekar *et al.*, 2018).

Interestingly, the remaining three isolates (SR1bi, SR5bi and SR10bi) recovered from infected tissues were segregated from the first subclade as they clustered forming a second cluster or subclade together with *Dickeya* species. Most of the *Dickeya* species has been reported to cause serious soft rot diseases in crops, fruits and ornamental plants. As a group, *Dickeya* species are considered as one of the most important bacterial phytopathogens (Mansfield *et al.*, 2012). Most species of *Dickeya* have a wide host range and infection can be initiated in dicotyledon and monocotyledon (Hussain *et al.*, 2008). Infections of Dickeya can be characterised as invasion at the center of the rhizome, which results in maceration, rotting and vascular discoloration (Perombelon and Kelman, 1980). One of the *Dickeya* species in the cluster is *D. dadantii* or previously known as *E. chrysanthemi* can cause soft rot diseases on many crops. These bacteria have the ability to survive in soils, and the mode of transmission to plants is via water, insects, or cultural technique. The common symptom for *D. dadantii* infection, or identified as pectinolytic soft rot *Erwinia*, is characterised by the disruptions of parenchymatous tissues, caused by pectic enzymes (Hugouvieux-Cotte-Pattat *et al.*, 1996). Nevertheless, soft rot *Erwinia* that colonizes plant involves a few cascading mechanism factors such as depolymerisation of cellulase that can cause degradation of plant cell wall components, assimilation of Ferum, an Hrp type III secretion system, exopolysaccharides, motility and plant defence protein (Toth *et al.*, 2003). An interesting finding from the generated phylogenetic study herein shows that apart from *Erwinia* sp. that has been reported as the most frequently isolated bacterial strains from necrotic lesion on leaves and rachises (Monge Pérez *et al.*, 1993), *Klebsiella* sp. may also be another bacterial strain potential in causing the disease. This observation and isolation of *Klebsiella* sp. is the first to be reported for CSR in oil palm.

Most of the bacterial isolates are related to soft rot disease suggesting that the isolates are highly potential in initiating CSR disease in oil palms. However, this warrants for pathogenicity tests using individual bacterial and fungal isolates.

CONCLUSION

A number of fungal and bacterial isolates were successfully isolated from the CSR oil palm infected tissues. All the isolates are expected to be associated with CSR disease as some of the fungal species have been reported earlier to cause disease in other host. This study also reports the first findings of *Klebsiella* sp. as one of the most frequently isolated bacterial strain in this study. Nevertheless, a follow up study is crucial in determining the causal agent of CSR disease in palms by conducting a Koch's postulate investigation using the isolated fungal and bacterial collection.

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