Journal of Plantation Crops 2026, 54(1): 30-41
https://doi.org/10.25081/jpc.2026.v54.i1.9960

Research Article

Characterization of pink disease isolates, *Erythricium salmonicolor*, from cocoa and *in vitro* management of the disease

Patrick Enchill*

Kwame Nkrumah University of Science and Technology, Kumasi, Ashanti Region, Ghana

(Received: April 17, 2026; Revised: July 10, 2026; Accepted: July 16, 2026)

*Corresponding author: Patrick Enchill, E-mail: patrickenchill@outlook.com

Abstract

Pink disease, caused by *Erythricium salmonicolor,* affects cocoa production in Ghana. This study aimed to characterize *E. salmonicolor* isolates from five cocoa-growing communities in Ghana and evaluate disease management strategies in vitro in vitro. The isolates were evaluated using morphological and molecular (ITS-PCR) identification, growth profiling across eleven media under different temperatures (25-35 °C) and light regimes, vegetative compatibility assays, pathogenicity tests, and in vitro fungicide sensitivity. Isolates exhibited variations in growth, texture, and pigmentation, with three color groups. All isolates produced hyaline hyphae with clamp connections. ITS-PCR produced 750-800 bp fragments. Maximum growth occurred on PDA and MEA at 28 °C. Light was essential for pink pigmentation. Vegetative compatibility revealed both compatible and incompatible reactions. Three isolates infected unwounded cocoa seedlings. Ridomil Gold and Cabrio Duo achieved 100% inhibition of all isolates at all concentrations, while Banjo Forte was least effective. Diverse *E. salmonicolor* strains exist in Ghana. Cabrio Duo offers an effective non-copper fungicide alternative.

Keywords: *Corticium salmonicolor*, Copper fungicides, Disease management, Ghana, Pink disease, Seedlings

Introduction

Ghana is the world's second-largest cocoa (*Theobroma cacao* L.) producer, with an export value of US$1.08 billion. Cocoa is a major source of foreign exchange and supports over 800,000 smallholder farmer households (Kongor *et al.,* 2024). It provides the primary source of income for about 25% of the population (Adams and Carodenuto, 2023) and plays a vital role in reducing rural poverty (Kumi & Daymond, 2015). Ghanaian cocoa is internationally recognized for its superior bean quality, largely attributed to its unique fermentation process (Ghana Cocoa Board, 2022). However, cocoa production is constrained by several fungal diseases that threaten sustainable yield.

Among these diseases, black pod caused by *Phytophthora* spp. is the most economically important, causing approximately 10% annual tree mortality and 20–30% yield losses worldwide (Akrofi *et al.,* 2016; Yara, 2018). Other fungal diseases include anthracnose (*Colletotrichum gloeosporioides*), white thread blight (*Marasmius tenuissimus*), stem cankers (*Phytophthora palmivora*) (Amoako-Attah *et al.,* 2016; Asare *et al.,* 2021; Second *et al.,* 2021), and pink disease caused by *Erythricium salmonicolor* (Berk. & Broome) (syn. *Corticium salmonicolor*, *Phanerochaete salmonicolor*), a basidiomycete characterized by hyaline, thin-walled hyphae with clamp connections (Kwarteng *et al.,* 2018).

E. salmonicolor has a wide host range, infecting economically important crops including rubber (*Hevea brasiliensis*), cocoa, coffee, citrus, mango, and eucalyptus (Kwarteng *et al.,* 2018). In Ghana, pink disease was first reported in experimental cocoa plots at Busno in the Eastern Region and has since spread to major cocoa-growing areas including the Eastern, Ashanti, Ahafo, and Western regions (Akrofi *et al.,* 2014, 2016; Kwarteng *et al.,* 2018). The pathogen infects through wounds or directly penetrates bark, killing the cambium (Roux & Coetzee, 2005; Sebastianes *et al.,* 2007). Typical symptoms include gum exudation, white mycelial growth that later develops into pink to salmon-colored mycelium, branch dieback, fruit drop, and reduced plant growth (Akrofi, 2016; Compendium, 2019). Severe infections of the main stem can kill entire trees (Akrofi, 2016; Government of Queensland, 2024). The pathogen develops four distinct growth stages—cobwebby mycelium, pink to salmon-colored incrustation, creamy pustules, and orange fruiting bodies—depending on environmental conditions and disease progression (Akrofi *et al.,* 2014; Akrofi, 2016; Government of Queensland, 2024).

Despite its destructive potential, pink disease has received considerably less attention than black pod disease. Although morphological characterization of isolates from the Eastern Region has been reported (Kwarteng *et al.,* 2018), little is known about the diversity of *E. salmonicolor* populations across Ghana's cocoa-growing regions or whether distinct strains exist with differing phenotypic and genotypic characteristics. Understanding pathogen diversity is important because it may influence virulence, host specificity, and responses to disease management strategies (Corredor-Moreno & Saunders, 2020).

Management of pink disease in Ghana relies mainly on copper-based fungicides and copper–metalaxyl mixtures (Akrofi, 2016). However, prolonged use of copper fungicides can promote resistance and negatively affect the environment and biodiversity (Pesce *et al.,* 2025). Furthermore, fungicide efficacy declines once infections become established, emphasizing the need for integrated management combining sanitation, pruning, and appropriate fungicide application (Akrofi *et al.,* 2014; Akrofi, 2016).

Evaluating alternative fungicides with different modes of action is therefore warranted. In addition, vegetative compatibility analysis provides valuable insights into population structure and genetic relatedness. Studies from other countries have shown heterogeneity among *E. salmonicolor* isolates based on heterokaryon formation (Sebastianes *et al.,* 2007), but comparable information is lacking for Ghanaian cocoa-growing regions. This knowledge gap limits understanding of pathogen movement, gene flow, and strain differentiation. Therefore, this study aimed to identify and characterize *E. salmonicolor* isolates from major cocoa-growing districts of Ghana by confirming their identity, assessing isolate variability, determining vegetative compatibility, and evaluating the effectiveness of selected fungicides against their growth.

Methodology

Fungal isolation and maintenance

Study area and sample collection

The study was conducted at the Mycology Laboratory of the Plant Pathology Division and the greenhouse of the Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim, in the Eastern Region of Ghana (latitude 6.23° N, longitude 0.36° W). Five cocoa farms in four cocoa-growing locations across the Eastern Region (Fanteakwa, Osino, and Samaang; East Akim, Busno), Western North (Sefwi-Wiawso, Ayisakrom), and Western Region (Jomoro, Elubo) of Ghana, where the disease has been previously reported, were surveyed and sampled. Diseased cocoa branches showing characteristic salmon-colored encrustation were collected using a sharp machete and placed into sterile zip-lock polythene bags. Samples were transported to the laboratory in a cooler containing ice packs to maintain viability. To prevent cross-contamination between farms, all sampling tools were sterilized with 70% ethanol before and after each collection.

Isolation and pure culture establishment

Pink mycelia and incrustation from infected twigs were used for direct isolation. Sterilized tissue samples were aseptically transferred onto water agar plates and incubated at 28±1 °C for three days with a 12-hour photoperiod (12 h light: 12 h Darkness) to obtain pure cultures.

Hyphal tips from actively growing colonies were excised under a stereo microscope (Leica Microsystems, Wetzlar, Germany) using a sterile fine-point needle and transferred to fresh potato dextrose agar (PDA) plates. This process was repeated to ensure cultural purity. A 5 mm diameter cork borer was used to extract mycelia plugs from the margins of 7-day-old fungal cultures for subsequent experiments. Each isolate was replicated three times and incubated at 28±1 °C in a completely randomized design. Five pure cultures (Elubo, Saamang, Ayisakrom, zOsino, and Bunso) were obtained and maintained on PDA slants at 4 °C for short-term storage.

Morphological characterization

Culture media preparation

Eleven different culture media were used, categorized as poor media, general-purpose media, rich media, and cocoa-part amended media (Table 1). The various media compositions were adopted from different sources and modified to suit the purpose of this study. Cocoa-part amended media was prepared using protocols described by Awuah and Frimpong (2002). All media were autoclaved at 121 °C for 15 minutes, with pH adjusted between 5.5 and 6.0 before autoclaving (Stevens, 1981). Media were poured into 90 mm sterile Petri dishes (15-20 mL per plate) in a laminar flow hood.

Growth and colony characteristics

Using the same inoculation procedure described earlier, mycelial plugs were transferred onto each of the eleven-test media and incubated at 28±1°C in darkness for seven days (Tuite, 1969). Colony diameter and growth rate (mm/day) were measured at 24-hour intervals for seven days. Colony color, texture, form, elevation, and margin characteristics were recorded. Colony surface coloration was described using the description of Shrestha *et al.* (2006). The key prescribed by Leung and Liu (2016) was used to describe

Table 1: Culture media used for morphological characterization of *E. salmonicolor* isolates

Medium category	Medium (Abbreviation)	Preparation method	Source
Poor media	Water Agar (WA)	20 g agar powder in 1 L of distilled water	Stevens, 1981
General-purpose media	Malt Extract Agar (MEA)	50 g OXOID MEA in 1 L distilled water	Atlas, 1993
	Sabouraud Dextrose Agar (SDA)	Prepared according to the manufacturer's protocol	Atlas, 1993
	Corn Meal Agar (CMA)	Prepared according to the manufacturer's protocol	Johnston & Booth, 1983
	V-8 Juice Agar (V8)	Prepared according to the manufacturer's protocol	Johnston & Booth, 1983
Rich media	Potato Dextrose Agar (PDA)	39 g OXOID PDA in 1 L distilled water	Johnston & Booth, 1983
	Nutrient Agar (NA)	Prepared according to the manufacturer's protocol	Atlas, 1993
	Yeast Extract Agar (YEA)	Prepared according to the manufacturer's protocol	Tuite, 1969
Cocoa-part amended media	Cocoa Bark Agar (CBA)	90 g blended cocoa bark, filtered, topped to 500 mL, 10 g agar	Awuah & Frimpong, 2002
	Green Cocoa Pod Husk Agar (GCPHA)	90 g macerated pod husk, filtered, topped to 500 mL, 10 g agar	Awuah & Frimpong, 2002
	Cocoa Juice Agar (CJA)	200 mL cocoa pulp juice, topped to 500 mL, 1.25 g $CaCO_3$, 10 g agar	Awuah & Frimpong, 2002

colony morphology, including form, elevation, and margin characteristics.

Mycelial density was visually classified using the "comparative description" method from Shrestha *et al.* (2006). Depending on the density of aerial mycelia, mycelia density was visually classified as poor (0.0), moderate (0.2-0.4), and abundant (>0.4). Extremely low mycelia density was designated as 0.0. Similarly, the mycelial texture was classified as felty, cottony, and fluffy, while the extremely poor texture was categorized as flat.

Molecular characterization

DNA extraction

Total genomic DNA was extracted from 8-day-old fungal mycelia grown on malt extract agar (MEA) plates at a temperature of 28 ± 1°C. The mycelia were harvested by scraping the fungal colonies with a sterile scalpel and transferred to a sterile conical flask. The modified DNA extraction protocol of Al-Samarrai and Schmid (2001) was used.

Polymerase Chain Reaction (PCR) amplification

PCR was carried out by targeting the ribosomal DNA (rDNA) with the universal primers ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') as the forward primer and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') as the reverse primer, described by White *et al.* (1990), were used. PCR amplification was performed in a total reaction volume of 25 µL reaction containing 12.5 µL of 2X master mix, 1 µM of ITS1 primer, 1 µM of ITS4 primer, and 1.0 uL of template DNA, and nuclease-free water to adjust the final volume. The amplification was conducted in a thermocycler with an initial denaturation of 94 °C for 2 mins, followed by 35 cycles of 20 s at 94 °C, 30 s at 53 °C, 30 s at 72 °C, and a final extension step at 72 °C for 5 mins.

Electrophoresis and visualization

All amplified PCR products were centrifuged briefly after being added to 2 µL of bromophenol blue and allowed to cool. Eight microliters (8 µL) of each PCR product were loaded into separate wells in a 1.0% agarose gel in 1 X TAE buffer, stained with bromophenol blue. Five microliters of PCR Ranger 50bp DNA ladder ((Norgen Biotek Corp., Thorold, ON, Canada)) (50 bp - 1000 bp) were also loaded into a separate well to serve as a marker. PCR products were run for an hour at 120 V, 100 mA, and 50 W in an electrophoretic chamber (EV231, Consort BVBA, Turnhout, Belgium) (Consort power system).

All DNA bands were visualized under UV light by transferring the agarose gel onto a High-Performance Ultraviolet Trans illuminator (UVP Benchtop Transilluminator, Analytik Jena, Jena, Germany). A camera was then used to record the image.

Temperature and light experiments

Temperature treatments

Mycelial agar plugs were taken from the edge of an actively growing, 7-day-old colony plate of pure cultures of each of the isolates using a sterile cork borer of 5 mm diameter. The mycelial plug was placed in the center of Petri dishes containing PDA. The inoculated plates were incubated under four different temperatures: 25 °C, 28 °C, 30 °C, and 35 °C. Data was taken on colony diameter, rate of growth, and sporulation daily for 7 days. This was done to assess the effect of temperature on the growth of *E. salmonicolor*. There were three replications per isolate.

Light regime treatments

To determine the effect of light on mycelial growth and pigmentation, inoculated PDA plates were incubated under three different light regimes at 28±1 °C. For continuous light exposure, one set of plates was exposed to fluorescent light for 20 days. For total darkness, a second set was wrapped with aluminum foil and placed in a sealed plastic tray for 20 days. For alternating light and dark conditions, a third set was covered in aluminum foil and kept in a sealed tray for 12 hours before being transferred to an incubation chamber with 12-hour photoperiods for 20 days.

Sporulation induction

The division Basidiomycetes to which the pathogen belongs does not sporulate readily on artificial agar medium. The experiment was aimed at determining the circumstances that could trigger the sporulation of the cocoa pathogen, *E. salmonicolor*. Isolates inoculated on the different media, including the cocoa-amended media, as well as under different temperatures, were checked for sporulation after 20 days of inoculation.

Malt extract agar (MEA) was poured into Petri dishes, and sterilized twigs of cocoa (autoclaved) were placed on the agar. Mycelium agar plugs (10 mm diameter) obtained from pure cultures of each isolate were used to inoculate the plates. Sporulation was observed after 20 days. Five-day intervals were used to check cultures for sporulation, but 20 days after inoculation were needed to observe any sporulation structures.

Vegetative compatibility analysis

Vegetative compatibility between isolates from different cocoa growing areas was determined by pairing isolates on PDA medium. Isolates used in crossing were labeled as Bunso (B), Saamang (S), Ayisakrom (A), Osino (O), and Elubo (E).

Mycelial plugs (5 mm diameter) taken from the margins of actively growing 7-day-old cultures of each isolate were placed approximately 3 cm apart on 90 mm PDA plates. Each pairing was performed in duplicates. Plates were incubated at 28±1 °C and observed for 15 days.

Hyphal interaction was assessed both macroscopically and microscopically. While acroscopic observations were recorded for the presence or absence of a barrage line or clear zone between paired colonies, microscopic observations encompassed mycelial samples taken from the interaction zone, mounted on glass slides, and examined under a light microscope for hyphal fusion events.

Following these observations, pairing was scored as compatible (C+) when hyphal contact and fusion were observed, or incompatible (C-) when no hyphal fusion occurred, even when there was a hyphal contact.

In Vitro fungicide sensitivity testing

Fungicides tested

Five different fungicides according to their recommended dosages by CRIG for cocoa black pod disease (Drenth & Guest, 2016.) were utilized in vitro on the fungal isolates (Table 2).

Fungicide preparation and poisoned food technique

Fungicides were evaluated at four distinct proportions (25%, 50%, 75%, and 100%) of approved dosages of each fungicide were used. The calculated concentration of each fungicide was amended with 300 mL of prepared PDA using the poisoned food technique. Each fungicide concentration was computed using the mass-by-volume equation adapted from standard agrochemical dilution protocols (Dhingra & Sinclair, 1985):

$$\text{AD (g)} = RD\ (g) \times (mL) \times 100/RV(mL) \text{ x } 100$$

where AD=Application dosage weight (g), RD=Recommended dosage weight (g), AV=Constant application volume (mL), RV=Recommended volume (mL).

These calculations were made for each fungicide concentration using C1V1=C2V2 (Harris, 2010) where: C_1=recommended concentration, V_1=the volume used on the field, C_2=the final solution, and V^2=the final volume.

The fungicide amended with PDA was poured into a heat-sterilized plate and allowed to solidify. Pure cultures inoculated on PDA without any fungicides served as control.

Table 2: Fungicides, active ingredients, and recommended application rates

Fungicide	Active ingredient(s)	Recommended dosage (per 15 L of water)
Ridomil Gold Plus	6% Metalaxyl-M + 60% Copper	50 g
Metalm	120 g/kg Metalaxyl + 600 g/kg Copper oxide	75 g
Forum R WP	60 g/kg Dimethomorph + 400 gm/kg Copper	75 g
Banjo Forte SC 400	200 g/L Dimethomorph + 200 g/L Fluazinam	75 mL
Cabrio Duo	40 g/L Pyraclostrobin + 72 g/L dimethomorph	50 mL

Inoculation and growth inhibition assessment

Agar plates were inoculated with a 5 mm-diameter disc of mycelia taken with a flame-sterile cork borer from the growing margins of one-week-old cultures of the various isolates. Inoculated plates were incubated for five days at 28±1 °C in the incubation chamber to ensure uniform thermal conditions across replicates. Two perpendicular straight lines were drawn along the diameter of each Petri dish. The mycelial growth was measured using a ruler on the bottom side of the Petri dish five days after inoculation.

Percentage inhibition of mycelia growth (I) was calculated using Vincent's formula as used by Gupta and Tripathi (2011):

$$I = [(Dc - dT)/DC] \times 100\%$$

Where I=Percentage inhibition, Dc=Mean radial growth diameter in control plate, dT=Mean radial growth diameter in treated plate.

Each fungicide concentration was tested in triplicate for each isolate.

Pathogenicity assay on cocoa seedlings

Seedling preparation

Topsoil from a forest area in CRIG was thoroughly sieved to remove stones, plastic materials, and plant debris. To reduce initial microbial loads, the soil was solarized by exposure to direct sunlight for 48 hours; while autoclave pasteurization is standard, solarization was selected due to bulk soil volume constraints and its common application in regional nursery setups. The soil was then sieved and dispensed into 30 plastic nursery bags. Each nursery bag contained 1500 mL of soil. After filling the bags with soil, they were watered and allowed to drain for three hours.

Thirty mixed-hybrid (Series II) cocoa seedlings were raised in nursery bags with one seedling per bag to reduce overcrowding and competition. Watering of the bags was done at two-days interval. The established cocoa seedlings were arranged in six groups, with each group consisting of individuals to be inoculated with isolates from a particular cocoa-growing district, and one group representing the control.

Inoculum preparation

The cocoa seedlings were inoculated using infected cocoa twigs. Six 9cm long cocoa twigs were autoclave sterilized and dipped in a beaker with 20 mL MEA. The MEA with the cocoa twigs was inoculated with a 1 cm diameter of mycelial discs of one-week-old culture growth from the various districts. The MEA with the cocoa twigs was sealed using aluminum foil and incubated at 28 °C for

four days. After four days, the cocoa twig showed signs of mycelial growth.

Inoculation and disease assessment

The seedlings were divided into two groups: wounded and unwounded plant stems. The surfaces of the stems of individual plants were sterilized with 70% ethanol before inoculation. A sterile surgical blade was used to create a wound on the stem of the seedlings for the wounded treatment. The stems were inoculated with the infected twigs of the isolates. In order to ensure good contact between the cocoa seedling and the inoculum and a high infection rate, inoculation was carried out by taping the twigs (the inoculum) to the stem and holding them in place with moistened cotton wool. Inoculated plants were sprayed with distilled water to provide a good ambient humidity for infection. The setup was maintained in the Plant House of the Cocoa Research Institute of Ghana under natural ambient daylight conditions (approximately a 12-h photoperiod), with a mean ambient temperature of 29±2 °C and a relative humidity range of 75%-85% throughout the experimental period. Five plants that were not inoculated were used as controls.

The lesion growth rate was measured fifteen days after inoculation. Samples taken from the infected parts were used for re-isolation and examination in the laboratory. The re-isolated pathogens were then compared with the previously isolated ones to fulfill Koch's postulates.

Statistical analysis

The collected data were subjected to ANOVA using the Genstat statistical package (Payne *et al.,* 2009). ANOVA revealed a significant difference in means ($P \leq 0.05$), the least significant difference (LSD) at 5% was used to separate means in fungal radial growth on different media.

Results

Isolation and cultural characteristics

Pink disease pathogen was isolated from diseased cocoa twigs collected from five farms in the Eastern, Western North, and Western Regions of Ghana. Five isolates were obtained and labeled according to their specific locations: Elubo, Saamang, Ayisakrom, Osino, and Busno (Table 3).

On PDA medium, all five isolates produced distinct colony characteristics (Table 3). The cultures had a predominant circular form and were mostly raised, exhibiting margins that were either smooth and regular (entire) for the Osino, Elubo, Saamang, and Ayisakrom isolates, or thread-like and irregular (filiform) for the Bunso isolate. The Osino and Elubo isolates produced fluffy and cottony textures, while the Bunso, Saamang, and Ayisakrom isolates showed a flat leathery texture.

Different shades of pink coloration were observed both on the surface and on the reverse side of PDA plates for all isolates (Table 3). The Osino isolate formed a yellow exudate on the colony surface. The Elubo isolate initially appeared cottony-white with thread-like mycelia at the advancing margins but turned pinkish-white after ten days of incubation. Based on pigmentation on PDA, the isolates were separated into three groups: deep salmon pink (Bunso), salmon pink (Osino, Saamang, Ayisakrom), and whitish pink (Elubo).

Mycelial growth rates varied among isolates (Table 3). The fast growing, The fast-growing Elubo and Osino isolates completely covered the 90 mm Petri dishes within 4 to 5 days of incubation, whereas the slow-growing Bunso isolate required 8 to 9 days to achieve full plate coverage. PCR amplification of total genomic DNA using primer pair ITS1–ITS4 produced a single PCR product of approximately 750- 800 bp for all isolates (Figure 1a), consistent with the expected size for *E. salmonicolor*.

Microscopic examination revealed that all isolates produced hyaline, thin-walled, and non-septate hyphae with characteristic clamp-connections (Figure 1b), confirming their placement in the Phylum Basidiomycota. No sporulating structures or basidiospores were observed on any of the isolates.

Effect of culture media on growth

All eleven-culture media used supported the growth of *E. salmonicolor* to varying degrees. Maximum growth on PDA, SDA, V8, and MEA for isolates from Elubo, Osino, and Samang (Figure 2). CBA and GCPHA also supported maximum growth for Osino and Elubo isolates. CJA supported the least growth among all five isolates. Isolates from Bunso and Ayisakrom consistently showed slower growth compared to the other isolates across all media tested.

On PDA, the mean colony diameter of the isolates after six days was: Elubo (64 mm), Osine (60.92 mm), Ayisakrom (54 mm), Samaang (43.3 mm), and Busno (36.9 mm). V8 juice agar, CBA, and GCPHA also supported mycelial growth to a maximum degree for most isolates. WA, YEA,

Table 3: Cultural and morphological characteristics of *Erythricium salmonicolor* isolates from five cocoa-growing districts in Ghana

Isolate	Source Location	Colony Color	Texture	Form	Elevation	Margin	Growth Rate
Bunso	Bunso, Eastern Region	Deep salmon pink	Flat leathery	Filamentous	Flat	Filiform	Slow
Osino	Osino, Eastern Region	Salmon pink	Fluffy	Circular	Raised	Entire	Fast
Saamang	Saamang, Eastern Region	Salmon pink	Flat leathery	Circular	Flat	Entire	Moderate
Ayisakrom	Ayisakrom, Western North	Salmon pink	Flat leathery	Circular	Raised	Entire	Moderate
Elubo	Elubo, Western Region	Whitish pink	Cottony	Circular	Raised	Entire	Fast

Characteristics observed on Potato Dextrose Agar (PDA) after 7-10 days of incubation at 28°C. Growth rate classification: Slow (<5 mm/day), Moderate (5-10 mm/day), Fast (>10 mm/day) based on mean daily radial growth

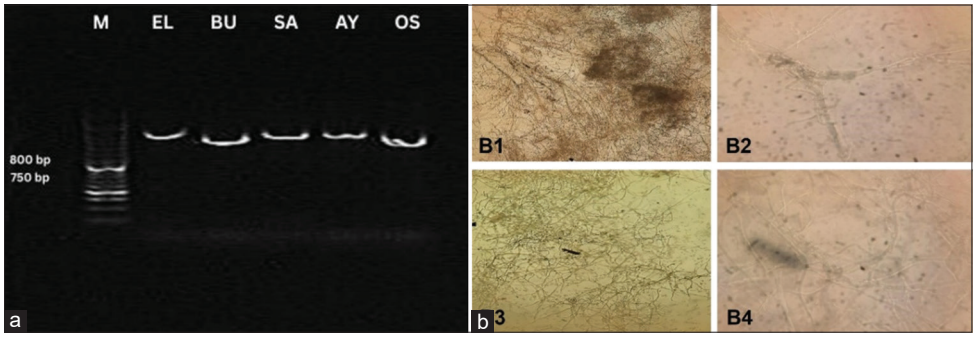


Figure 1: Molecular and microscopic characterization of *Erythricium salmonicolor* isolates from cocoa in Ghana. (a) PCR amplification of the ITS region using primers ITS1 and ITS4. Lane M: DNA ladder; EL: Elubo isolate; BU: Bunso isolate; SA: Saamang isolate; AY: Ayisakrom isolate; OS: Osino isolate. (b) Light micrographs of E. salmonicolor hyphae showing characteristic clamp connections. B1: Osino isolate (X400). B2: Osino isolate (X100). B3: Saamang isolate (X400). B4: Saamang isolate (X100)

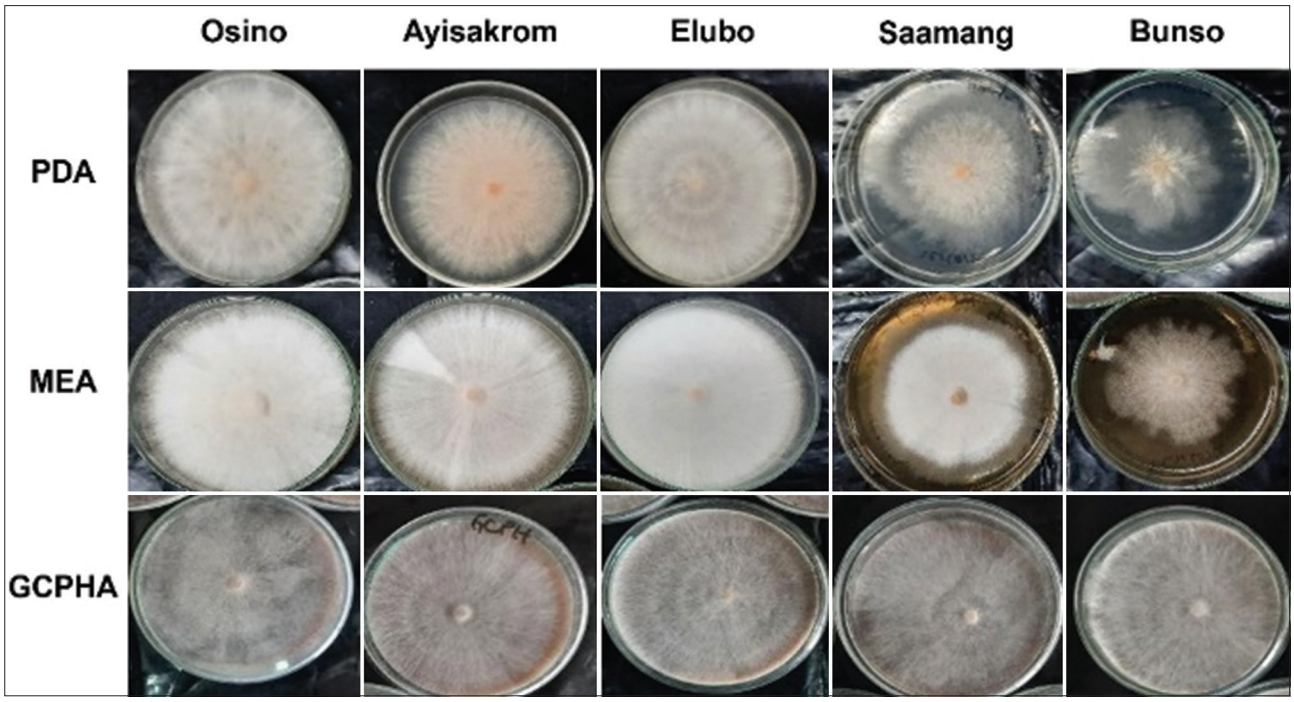


Figure 2: Growth of *Erythricium salmonicolor* isolates on three representative culture media after six days at 28 °C. Top row: Potato Dextrose Agar (PDA). Middle row: Malt Extract Agar (MEA). Bottom row: Green Cocoa Pod Husk Agar (GCPHA). From left to right in each row: Osino, Ayisakrom, Elubo, Saamang, Bunso isolates

NA, and CJA resulted in poor growth with small colony diameters for all isolates.

Color variations were observed across different media for each isolate (Figure 2). The Elubo isolate showed whitish coloration on most media, with pinkish-white coloration on SDA after six days. The Saamang isolate exhibited salmon-pink to pinkish-white coloration on PDA, SDA, MEA, NA, and CJA. The Bunso isolate showed pinkish-white coloration on the surface of PDA medium with salmon pink on the underside, while creamy coloration was observed on SDA and MEA surfaces with pinkish-white underside. The Ayisakrom isolate produced strong salmon-pink to pinkish-white coloration on both the surface and underside of PDA, SDA, MEA, and CJA. The Osino isolate showed a pinkish-white coloration on the surface of PDA, SDA, and MEA, with pinkish-white on the underside of NA.

For all the isolates, a whitish coloration was observed on the surface of V8, CBA, and NA media. CMA and WA produced colorless mycelia on all isolates except Bunso, where CMA gave white coloration on the surface.

Effect of temperature and light on growth

Temperature significantly influenced the growth and pigmentation of *E. salmonicolor* isolates (Table 4). The optimum growth temperature for all isolates was 28 °C, with growth declining at 30 °C and severely restricted at 35 °C. At 25 °C, growth rates were generally reduced compared to 28 °C.

At 25°C, salmon pink coloration was observed on both the surface and the underside of PDA plates for most isolates after six days of incubation under alternating light and dark conditions (12 h light: 12 h darkness)

At 28 °C, all isolates showed enhanced growth with characteristic pigmentation (Figure 3). Color variations were observed on the reverse side of plates, with Saamang and Ayisakrom isolates showing salmon pink coloration, while the Elubo isolate showed whitish coloration that turned pinkish-white after 15 days of inoculation.

At 30 °C, salmon-pink coloration was observed on the surface of Osino, Ayisakrom, Saamang, and Bonsu isolates, while the Elubo isolate produced whitish coloration after six days. Creamy coloration was observed on the underside of Ayisakrom, Saamang, and Bonsu isolates. Growth rates were reduced at this temperature, with the Ayisakrom isolate showing the least growth, 14.2 mm/day.

Light regimes markedly affected mycelial pigmentation (Table 5 and Figure 4). Under continuous light and alternating light/dark conditions, all five isolates developed salmon pink to deep salmon pink coloration. Under total darkness, all isolates produced white mycelia with no pink pigmentation. Mycelial density varied among isolates under all conditions, while Saamang consistently showed poor mycelial density. All isolates showed a fluffy and cottony texture except the Saamang, which maintained a flat mycelial texture under all the conditions (Table 5).

Table 4: Mean colony diameter (mm/day) of *Erythricium salmonicolor* after six days of incubation at different temperatures

Temperature	Bunso	Elubo	Saamang	Osino	Ayisakrom
25°C	25.8	51.8	44.6	54.0	43.9
28°C	36.8	64.8	43.3	60.9	54.1
30°C	26.2	37.3	28.1	38.0	14.2

Table 5: Mycelial growth characteristics of *Erythricium salmonicolor* on PDA under different light regimes

Isolate	Complete Light		Complete Darkness		Alternating Light/Dark	
	Density*	Texture	Density*	Texture	Density*	Texture
Osino	+ + +	Fluffy	+ + +	Cottony	+ + +	Cottony
Bonsu	+ +	Cottony	+ +	Cottony	+ +	Cottony
Saamang	+	Flat	+	Flat	+	Flat
Elubo	+ + +	Fluffy	+ +	Fluffy	+ + +	Cottony
Ayisakrom	+ +	Fluffy	+ +	Fluffy	+ +	Fluffy

*Poor (+), Moderate (+ +), Abundant (+ + +)

Vegetative compatibility

Pairings of *E. salmonicolor* isolates on PDA revealed both compatible and incompatible reactions (Table 6). Of the eleven crosses evaluated, four pairs (Bunso – Osino, Bunso – Elubo, Saamang – Osino, and Osino – Elubo) showed hyphal contact and fusion, indicating vegetative compatibility. Macroscopic observation showed that these compatible pairings resulted in hyphal contact without a clear zone of separation after 15 days of inoculation.

The remaining seven crosses (Ayiskarom – Osino, Ayiskarom – Elubo, Bunso – Ayiskarom, Saamang – Elubo, Saamang – Ayiskarom, and Bunso – Saamang) showed hyphal contact but no fusion, with a clear line of separation visible between colonies, suggesting vegetative incompatibility. Microscopic examination of the interaction zones confirmed hyphal fusion in compatible crosses (Figure 5), with distinct clamp connections visible at fusion points. In incompatible crosses, hyphae grew adjacent to each other without anastomosis.

All crosses showed various shades of salmon pink coloration regardless of compatibility status. Crosses involving the Bunso isolate consistently exhibited slower growth compared to other pairings.

Sporulation induction

No sporulation was observed from any of the five isolates under any of the conditions tested (including different growth media, temperatures, or light regimes), even after 20 days of incubation. However, on cocoa twigs placed on MEA, orange outgrowths were observed on the Saamang isolate when observed under the microscope. No sporulating structures were observed on any of the other isolates.

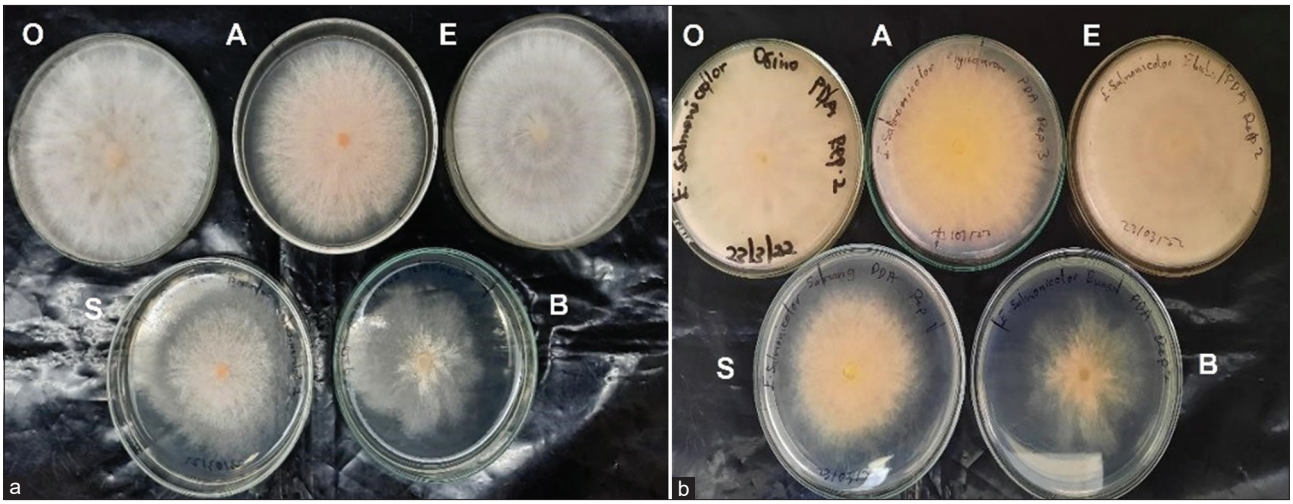


Figure 3: Growth of *Erythricium salmonicolor* isolates at optimum temperature (28 °C) after six days of incubation under alternating light/dark conditions. a) Colony surface view and b) Colony reverse view. Isolate labels: O = Osino, A = Ayisakrom, E = Elubo, S = Saamang, B = Bunso

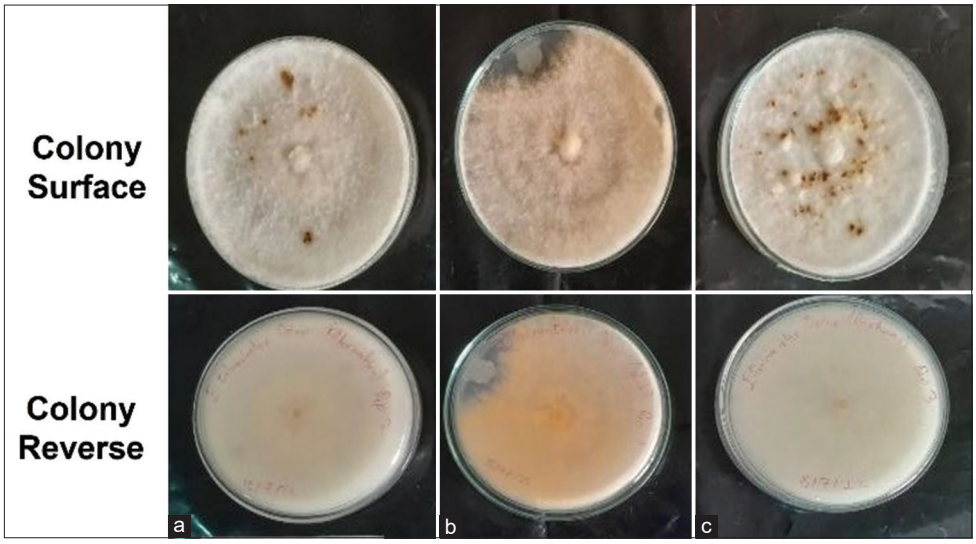


Figure 4: Effect of light regimes on mycelial pigmentation of *Erythricium salmonicolor* (Osino isolate) after 20 days of incubation. Top row: colony surface. Bottom row: colony reverse. (a) Alternating light/dark (12 h/12 h). (b) Continuous light. (c) Total darkness

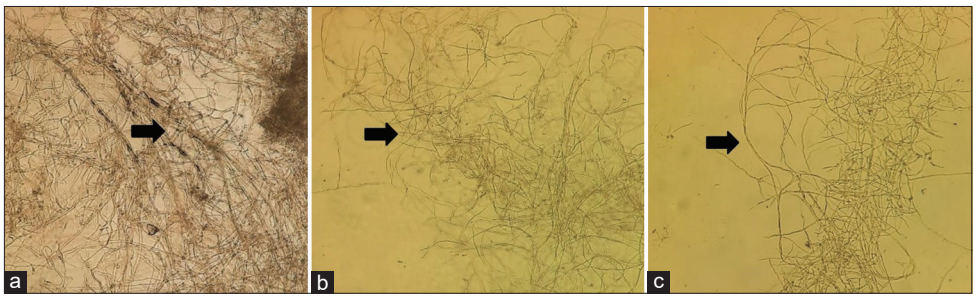


Figure 5: Microscopic evidence of hyphal fusion in compatible crosses of *Erythricium salmonicolor*. a) Hyphal fusion between Bunso and Osino isolates, b) Hyphal fusion between Bunso and Elubo isolates and c) Hyphal fusion between Saamang and Osino isolates. Arrows indicate points of fusion between hyphae

Table 6: Vegetative compatibility between isolates from different farms

Cross	Hyphal Interaction
Bunso – Osino	Compatible (C +)
Bunso – Elubo	Compatible (C +)
Saamang – Osino	Compatible (C +)
Osino – Elubo	Compatible (C +)
Ayisakrom – Osino	Incompatible (C-)
Ayisakrom – Elubo	Incompatible (C-)
Saamang – Elubo	Incompatible (C-)
Saamang – Ayisakrom	Incompatible (C-)
Bunso – Saamang	Incompatible (C-)
Bunso – Ayisakrom	Incompatible (C-)

C + = Presence of hyphal contact and fusion; C- = Absence of hyphal fusion

Pathogenicity on cocoa seedlings

All five isolates of *E. salmonicolor* successfully infected wounded cocoa seedlings, with infection rates varying among isolates (Table 7). On unwounded seedlings, three isolates (Elubo, Osino, and Ayisakrom) produced characteristic pink disease symptoms, while Bunso and Saamang isolates failed to infect the unwounded stems.

On wounded stems, Elubo, Osino, Saamang, and Ayisakrom isolates achieved 100% infection (3 out of 3 seedlings), while Busno achieved 67% infection (2 out of 3 seedlings). On unwounded stems, Elubo, Osino, and Ayisakrom achieved 100% infection, while Bunso and Saamang showed 0% infection.

Mycelial growth on infected seedlings varied among isolates (Table 7). The Elubo showed the most extensive mycelial growth on both wounded (10.0 mm) and unwounded (11.3mm) stems, followed by Osino (9.8 mm wounded, 10.0 mm unwounded). Busno showed the least growth (2.5 mm) and only on wounded stems. Control seedlings showed no infection or mycelial growth.

Initial symptoms appeared 10 to 14 days after inoculation as white mycelial fans on the stem surfaces. Over time, the white mycelia gradually turned pink, characteristic of *E.salmonicolor* infection. No symptoms developed on control seedlings (Figure 6).

Re-isolation from infected stem tissues yielded fungal cultures with morphological characteristics identical to the original isolates, fulfilling Koch's postulates.

Fungicide sensitivity

The five fungicides tested showed varying levels of inhibition against *E. salmonicolor* isolates. Ridomil Gold

and Cabrio Duo were significantly superior to all the other fungicides, achieving 100% inhibition of all five isolates at all concentrations tested (25%, 50%, 75%, and 100% of the recommended dosage) (Figure 7).

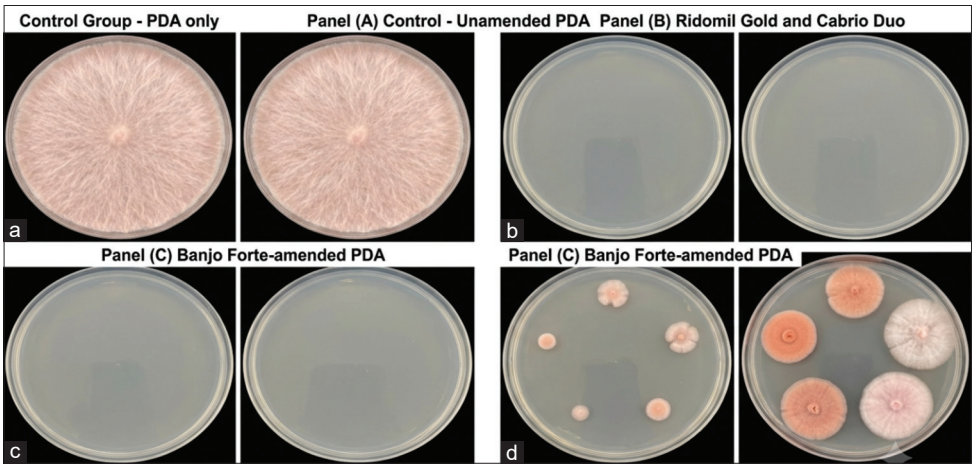
Mefenoxam achieved 100% inhibition at 50%, 75%, and 100% concentrations for all isolates, with slightly reduced inhibition at 25% for some isolates. Metalaxyl achieved 100% inhibition at 75% and 100% concentrations for all isolates, with variable inhibitions at lower concentrations. Fluazinam + dimethomorph was the least effective against, with inhibition ranging from 54-80% across isolates and concentrations.

In general, increasing fungicide concentrations resulted in a higher percentage inhibition of mycelial growth for all fungicides tested. Significant difference ($P \leq 0.05$) in fungicide sensitivity was observed among isolates. The Saamang, Osino, Ayisakrom, and Bunso isolates were completely inhibited by 75 and 100% concentrations of Metalm, while the Elubo isolate required Ridomil Gold and Cabrio Duo fungicide at lower concentrations to achieve 100% inhibition.

Discussion

The morphological and molecular characterization of *Erythricium salmonicolor* isolates from five cocoa-growing districts in Ghana revealed considerable diversity among populations. Isolates from Osino, Saamang, and Ayisakrom produced the characteristic salmon-pink pigmentation but differed in colony morphology, with Osino exhibiting fast-growing fluffy mycelia and Ayisakrom producing slow, flat colonies. Such phenotypic variation is commonly associated with genetic diversity, environmental adaptation, or host specialization (Corredor-Moreno & Saunders, 2020). The similarity between Osino and Saamang isolates from the same district (Fanteakwa) suggests local dispersal through

Figure 6: Symptoms of pink disease on cocoa seedlings inoculated with *Erythricium salmonicolor* isolates. (a) Infected seedling (Osino isolate) showing white mycelial fans on stem surface, 14 days after inoculation. (b) Ayisakrom isolate: left (unwounded stem with no infection); right (wounded stem showing infection). (c) Control seedling (uninoculated) showing no symptoms



AQ1 **Figure 7:** *In vitro* sensitivity of *Erythricium salmonicolor* isolates to different fungicide concentrations (100% recommended field dose) after five days incubation at 28 ± 1 °C. The top row a) provides unamended control plates with full, uninhibited radial mycelial expansion (0% inhibition) for a complete visual baseline. b) The middle row displays complete radial growth inhibition (100%) observed on PDA amended with Ridomil Gold and Cabrio Duo, illustrating clear plates across all isolates. c) The bottom-left panel shows partial inhibition on Banjo Forte-amended PDA, illustrating reduced and varied colony development across isolates. The bottom-right panel provides a distinct morphological reference of the five characterized isolates on unamended PDA. Plates are 90 mm in diameter

Table 7: Pathogenicity of *Erythricium salmonicolor* isolates on six-month-old cocoa seedlings

Isolate	Wounded Stem Infection (%)	Unwounded Stem Infection (%)	Mean Mycelial Growth on Wounded Stem (mm)	Mean Mycelial Growth on Unwounded Stem (mm)
Bunso	67	0	2.5	0.0
Elubo	100	100	10.0	11.3
Saamang	100	0	7.6	0.0
Osino	100	100	9.8	10.0
Ayisakrom	100	100	5.8	5.7
Control	0	0	0.0	0.0

wind, rain, or contaminated farm equipment, whereas differences among geographically distant isolates indicate population structuring. These findings support observations by Kwarteng *et al.* (2018) that isolates from the same region tend to be morphologically and molecularly related.

The characteristic pink pigmentation on the reverse side of all cultures confirmed its diagnostic value, although variation in colour intensity (deep salmon pink in Bunso and pale pink in Elubo) further suggests strain differentiation. Microscopic examination consistently revealed hyaline hyphae with clamp connections, confirming the placement of the pathogen within Basidiomycota (Roux & Coetzee, 2005; Aanen *et al.,* 2023). The absence of sporulation under all culture conditions agrees with previous reports describing the difficulty of inducing sporulation in many basidiomycetes (Rungjindamai & Jones, 2024).

ITS amplification generated fragments of approximately 750-800 bp for all isolates, consistent with *E. salmonicolor* (Kwarteng *et al.,* 2018). Although Bunso and Ayisakrom isolates were identified as the same species, differences in band size, together with similarities between Bunso and Osino despite contrasting colony morphology, indicate that morphological similarity does not necessarily reflect genetic similarity, as reported for other fungal pathogens (Stengel *et al.,* 2022). The close molecular relationship between Bunso and Osino may also reflect movement of infected planting materials, emphasizing the importance of clean planting stock and good farm sanitation in limiting pathogen spread.

Culture medium significantly influenced fungal growth. PDA, MEA, SDA, and V8 juice agar supported rapid growth, whereas WA, YEA, NA, and CJA were less suitable. The superior performance of PDA and MEA agrees with previous reports (Kwarteng *et al.,* 2018) and likely reflects their favourable carbon and nitrogen composition, both of which are essential for fungal development (Schoder *et al.,* 2024). Strong growth on cocoa bark and green cocoa pod husk amended media further suggests adaptation to host-derived substrates, indicating that infected plant debris may serve as an important inoculum reservoir in cocoa farms.

Temperature strongly affected both growth and pigmentation, with 28 °C supporting optimal growth across all isolates, while growth declined at 25 °C and 30 °C and was completely inhibited at 35 °C. These results are consistent with reports that the optimum temperature for *E. salmonicolor* is 28-30 °C (Florina *et al.,* 2017). Light also influenced pigmentation, with alternating or continuous light inducing the characteristic salmon-pink colour, whereas complete darkness produced white mycelia. This response may reflect ecological functions such as UV protection or reproduction and agrees with reports linking sunshine to pink disease severity (Bandanaa *et al.,* 2021).

Vegetative compatibility tests revealed both compatible and incompatible interactions among isolates. Four pairings (Bunso–Osino, Bunso–Elubo, Saamang–Osino, and Osino–Elubo) showed hyphal fusion, indicating genetic relatedness and possible gene flow across geographically separated populations. Compatibility between Bunso and Elubo suggests long-distance pathogen movement, potentially through infected planting materials or farm equipment (Drenth & Guest, 2016). In contrast, seven incompatible pairings indicate substantial genetic heterogeneity, consistent with reports that *E. salmonicolor* populations often fail to form heterokaryons (Sebastianes *et al.,* 2007). The absence of a significant relationship between vegetative compatibility and geographical origin further supports the existence of multiple strains or independent evolutionary lineages within Ghana.

All isolates were pathogenic to cocoa seedlings. Elubo, Osino, and Ayisakrom infected unwounded seedlings, supporting the observation of Akrofi *et al*. (2014) that *E. salmonicolor* can penetrate intact bark through lenticels. In contrast, Bunso and Saamang infected only wounded seedlings, demonstrating differences in aggressiveness among isolates. Such variation could influence disease spread and severity under field conditions and highlights the importance of regular monitoring and timely disease management rather than relying solely on treatment of wounded trees.

The fungicide sensitivity results represent one of the most significant findings of this study. Ridomil Gold (metalaxyl + copper) and Cabrio Duo (pyraclostrobin + dimethomorph) completely inhibited mycelial growth of all isolates, even at 25% of the recommended field rate. The excellent performance of Cabrio Duo is particularly important because it provides a promising non-copper alternative for pink disease management. Since current management in Ghana relies heavily on copper-based fungicides, which raise concerns regarding environmental contamination and resistance development, these findings suggest that lower application rates and alternative fungicides could improve disease management while reducing environmental impacts. Nevertheless, field validation is required before recommending reduced application rates.

This study has several limitations. Fungicide evaluations were conducted in vitro, and efficacy under field conditions may differ because of environmental factors, fungicide degradation, and plant–pathogen interactions. Likewise, pathogenicity tests were performed on seedlings under greenhouse conditions and may not fully represent disease development on mature cocoa trees. Future research should incorporate multilocus phylogenetic analyses using ITS, LSU, translation elongation factor 1-alpha (tef1-α), and β-tubulin (tub2) genes to better resolve the genetic diversity of Ghanaian *E. salmonicolor* populations and their relationship with global isolates. Long-term field studies are also needed to validate fungicide performance and develop integrated management strategies combining sanitation, pruning, resistant varieties, and fungicide rotation.

Conclusion

This study characterized *E. salmonicolor* isolates from five cocoa-growing districts in Ghana and demonstrated

morphological, physiological, and vegetative compatibility differences, indicating the presence of diverse pathogen strains. The pathogen grew optimally at 28 °C, exhibited light-dependent pigmentation, and all isolates were pathogenic to cocoa seedlings, with some capable of infecting unwounded bark. Ridomil Gold (metalaxyl + copper) and Cabrio Duo (pyraclostrobin + dimethomorph) completely inhibited mycelial growth in vitro, highlighting Cabrio Duo as a promising non-copper alternative for pink disease management. Overall, these findings improve understanding of *E. salmonicolor* diversity in Ghana and provide a basis for integrated disease management strategies incorporating sanitation, monitoring, and effective fungicide use to reduce pink disease in cocoa production.

Author contributions

Patrick Enchill: conceptualization, data curation, methodology, investigation, data analysis, validation, visualization, writing of the first draft to final manuscript preparation.

References

Aanen, D. K., van't Padje, A., & Auxier, B. (2023). Longevity of fungal mycelia and nuclear quality checks: A new hypothesis for the role of clamp connections in dikaryons. *Microbiology and Molecular Biology Reviews, 87*(3), e00022-21. https://doi.org/10.1128/mmbr.00022-21

Adams, M. A., & Carodenuto, S. (2023). Stakeholder perspectives on cocoa's living income differential and sustainability trade-offs in Ghana. *World Development, 165*, 106201. https://doi.org/10.1016/j.worlddev.2023.106201

Akrofi, A. Y. (2016). *Pink disease of cocoa—Ghana: Erythricium salmonicolor*. PlantwisePlus Knowledge Bank, Pest Management Decision Guides. https://doi.org/10.1079/pwkb.20177800647

Akrofi, A. Y., Amoako-Attah, I., Acheampong, K., Assuah, M. K., & Melnick, R. L. (2016). Fruit and canopy pathogens of unknown potential risk. In B. A. Bailey & L. W. Meinhardt (Eds.), *Cacao diseases: A history of old enemies and new encounters* (pp. 61-83). Springer. https://doi.org/10.1007/978-3-319-24789-2_11

Akrofi, A. Y., Amoako-Attah, I., Assuah, M., & Kumi-Asare, E. (2014). Pink disease caused by *Erythricium salmonicolor* (Berk. & Broome) Burdsall: An epidemiological assessment of its potential effect on cocoa production in Ghana. *Journal of Plant Pathology & Microbiology, 5*(1), 215. https://doi.org/10.4172/2157-7471.1000215

Amoako-Attah, I., Akrofi, A. Y., Rashid, B. H., Mercy, A., & Kumi-Asare, E. (2016). White thread blight disease caused by *Marasmiellus scandens* (Massee) Dennis Reid on cocoa and its control in Ghana. *African Journal of Agricultural Research, 11*(44), 4462-4470. https://doi.org/10.5897/AJAR2016.11681

Asare, E. K., Domfeh, O., Avicor, S. W., Pobee, P., Bukari, Y., & Amoako-Attah, I. (2021). Colletotrichum gloeosporioides s.l. causes an outbreak of anthracnose of cacao in Ghana. *South African Journal of Plant and Soil, 38*(2), 107-115. https://doi.org/10.1080/02571862.2020.1863485

Bandanaa, J., Asante, I. K., Egyir, I. S., Schader, C., Annang, T. Y., Blockeel, J., Kadzere, I., & Heidenreich, A. (2021). Sustainability performance of organic and conventional cocoa farming systems in Atwima Mponua District of Ghana. *Environmental and Sustainability Indicators, 11*, 100121. https://doi.org/10.1016/j.indic.2021.100121

Compendium, C. C. P. (2019). *Cocoa pink disease*. CABI Compendium.

Corredor-Moreno, P., & Saunders, D. G. O. (2020). Expecting the unexpected: Factors influencing the emergence of fungal and oomycete plant pathogens. *New Phytologist, 225*(1), 118-125. https://doi.org/10.1111/nph.16007

Dhingra, O. D., & Sinclair, J. B. (1985). *Basic plant pathology methods*. CRC Press.

Drenth, A., & Guest, D. I. (2016). Fungal and oomycete diseases of tropical tree fruit crops. *Annual Review of Phytopathology, 54*, 373-395. https://doi.org/10.1146/annurev-phyto-080615-095944

Florina, D., Wahyuno, D., & Sisanto, S. (2017). Kepekaan Erythricium salmonicolor Penyebab Penyakit Jamur Upas pada Kemiri Sunan terhadap Suhu, Cahaya, dan Fungisida. *Jurnal Penelitian Tanaman Industri, 23*(3), 115-122.

Ghana Cocoa Board. (2022). *Our premium quality cocoa: The thick & thin to a taste of difference*. Retrieved from https://cocobod.gh/news/our-premium-quality-cocoa-the-thick-thin-to-a-taste-of-difference

Ghana Cocoa Board. (2024). Ghana cocoa specification. https://cocobod.gh/pages/ghana-cocoa-specification

Government of Queensland. (2024). Pink disease. Business Queensland. Retrieved from https://www.business.qld.gov.au/industries/farms-fishing-forestry/forests-wood/pests-diseases/trees-timber/pink-disease

Harris, D. C. (2010). *Quantitative chemical analysis*. (8th ed.). W. H. Freeman and Company.

Kongor, J. E., Owusu, M., & Oduro-Yeboah, C. (2024). Cocoa production in the 2020s: Challenges and solutions. *CABI Agriculture and Bioscience*, 5(1), 102. https://doi.org/10.1186/s43170-024-00310-6

Kumi, E., & Daymond, A. J. (2015). Farmers' perceptions of the effectiveness of the Cocoa Disease and Pest Control Programme (CODAPEC) in Ghana and its effects on poverty reduction. *American Journal of Experimental Agriculture, 7*(5), 257-274. https://doi.org/10.9734/AJEA/2015/16388

Kwarteng, F. G., Cornelius, E., Acquah, K. K., & Asare, E. K. (2018). Morphological and molecular identification of the fungus associated with pink disease of cocoa (*Theobroma cacao* L) in the Eastern Region of Ghana. *International Journal of Pathogen Research, 1*(1), 1-8. https://doi.org/10.9734/ijpr/2018/v1i11161

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B., & Soutar, D. M. (2009). *GenStat for Windows*. (12th ed.). VSN International.

Pesce, S., Mamy, L., Sanchez, W., Artigas, J., Bérard, A., Betoulle, S., Chaumot, A., Coutellec, M.-A., Crouzet, O., Faburé, J., Hedde, M., Leboulanger, C., Margoum, C., Martin-Laurent, F., Morin, S., Mougin, C., Munaron, D., Nélieu, S., Pelosi, C., & Leenhardt, S. (2025). The use of copper as plant protection product contributes to environmental contamination and resulting impacts on terrestrial and aquatic biodiversity and ecosystem functions. *Environmental Science and Pollution Research, 32*, 2830-2846. https://doi.org/10.1007/s11356-024-32145-z

Roux, J., & Coetzee, M. P. A. (2005). First report of pink disease on native trees in South Africa and phylogenetic placement of *Erythricium salmonicolor* in the homobasidiomycetes. *Plant Disease, 89*(11), 1158-1163. https://doi.org/10.1094/PD-89-1158

Rungjindamai, N., & Jones, E. G. (2024). Why are there so few Basidiomycota and basal fungi as endophytes? A review. *Journal of Fungi, 10*(1), 67. https://doi.org/10.3390/jof10010067

Schoder, K. A., Krümpel, J., Müller, J., & Lemmer, A. (2024).

Effects of environmental and nutritional conditions on mycelium growth of three Basidiomycota. *Mycobiology, 52*(2), 124-134. https://doi.org/10.1080/12298093.2024.2341492

Sebastianes, F. L. D. S., Maki, C. S., Andreote, F. D., Araújo, W. L., & Pizzirani-Kleiner, A. A. (2007). Genetic variability and vegetative compatibility of *Erythricium salmonicolor* isolates. *Scientia Agricola, 64*(2), 162-168. https://doi.org/10.1590/S0103-90162007000200009

Second, Z. M. A., Aoudou, Y., & Rene, B. M. (2021). Aggressiveness of the fungi responsible for pod rot in cropping systems based on cocoa trees (*Theobroma cacao* L.) in Cameroon. *American Journal of Agriculture and Forestry, 9*(3), 156-163. https://doi.org/10.11648/j.ajaf.20210903.19

Stengel, A., Stanke, K. M., Quattrone, A. C., & Herr, J. R. (2022). Improving taxonomic delimitation of fungal species in the age of genomics and phenomics. *Frontiers in Microbiology, 13*, 847067. https://doi.org/10.3389/fmicb.2022.847067

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315-322). Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yara. (2018). *Cocoa: Pest and diseases*. Yara International.

Author Query ???
AQ1: Kindly mention part label d in the figure caption