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MICROBIAL LIPASES AS POTENTIAL CANDIDATES FOR GREENER FINGERMARK VISUALIZATION TECHNOLOGIES ON WET NON-POROUS OBJECTS: A REVIEW

(Lipase Mikrob Sebagai Calon yang Berpotensi bagi Teknologi Pemvisualan Cap Jari yang Lebih Hijau di atas Permukaan Objek Tidak Berliang yang Basah: Satu Tinjauan)

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Abstract

Fingermarks remain as important individual characteristic evidence for identifying individuals during forensic investigations. However, the assessment of latent fingermarks can be challenging due to their hidden nature, necessitating the development of suitable visualization methods. Currently, the available methods for visualizing fingermarks on wet non-porous objects (e.g., Small Particle Reagent) contain hazardous and toxic chemicals. As such, the utilization of *Candida rugosa* lipase nanoconjugate for developing a greener forensic fingermark visualization technology for wet non-porous objects has been suggested. Notwithstanding, the utilization of other microbial lipases for the same purpose remains unreported. Considering such an aspect, reviewing the potential of the different microbial lipases as candidates for fingermark visualization technology proves relevant. Hence, this review article that accentuates the contextual importance of microbial lipases for greener fingermark visualization technology complying with the prevailing guidelines and its challenges and future insights for forensic investigations merits scientific and forensic considerations.

Keywords: forensic science, latent fingermarks, microbial lipase, *Candida rugosa, Rhizomucor miehei*

Abstrak

Cap jari merupakan bukti ciri individu yang penting dalam mengenalpasti individu semasa penyiasatan forensik. Namun, penilaian cap jari pendam adalah mencabar kerana sifatnya yang tersembunyi, memerlukan pembangunan kaedah pemvisualan yang sesuai. Pada masa ini, kaedah tersedia bagi pemvisualan cap jari pada objek berliang yang basah (contohnya Pembangun Fizikal dan Reagen Partikel Kecil) mengandungi bahan kimia berbahaya dan toksik. Justeru, penggunaan lipase *Candida rugosa* konjugatnano bagi membangunkan teknologi pemvisualan cap jari forensik yang lebih hijau untuk objek tidak berliang yang basah telah dicadangkan. Walau bagaimanapun, penggunaan lipase mikrob yang lain bagi tujuan yang sama masih tidak dilaporkan. Mengambil kira aspek berkenaan, tinjauan potensi pelbagai lipase mikrob sebagai calon untuk teknologi pemvisualan cap jari terbukti relevan. Oleh itu, artikel tinjauan ini yang menyerlahkan kepentingan konteks lipase mikrob sebagai teknologi pemvisualan cap jari yang lebih hijau dan mematuhi garis panduan lazim, serta cabarannya dan pandangan terkehadapan bagi penyiasatan forensik melayakkan pertimbangan saintifik dan forensik.

Kata kunci: sains forensik, cap jari pendam, lipase mikrob, *Candida rugosa, Rhizomucor miehei*

Introduction

Fingermark and forensic application

A world-renowned French criminologist Edmond Locard, postulated that every interaction results in the presence of identifiable evidence, commonly referred to as the Locard's exchange principle [1]. Consequently, offenders will inevitably leave trace evidence, like fingermarks, at crime scenes [2]. The admission of fingermarks as evidence in a court of law has been based on three key factors: (a) their unique characteristics, (b) their persistence over an individual's lifetime, and (c) the availability of a systematic classification of broad ridge patterns [3]. The initial premise asserts that each person (including those genetically identical) exhibits unique fingermarks [4]. The level of distinctiveness heavily depends on the minutiae's characteristics, identity, quantity, and relative positioning. This is evident in the meticulous assessment for comparing the known and unknown prints [5].

The fingermark ridges would remain the same for an individual throughout his/her entire life [4], if the individual does not sustain a deep-skin injury that causes a damage to the dermal papillae. Intriguingly, such an injury may result in the development of a new ridge characteristic that can prove evidentially helpful in the identification process [6]. Although the relatively "less sharp" fingermarks are commonly observed among elderly people, attributable to the skin's loss of elasticity, the general arrangement of friction ridge skin would not be affected [7]. In addition, it is important to note that fingermarks feature distinct ridge patterns that can be methodically categorized into three main types: loops, whorls, and arches (Figure 1). The global distribution of these patterns is estimated at approximately 60-65%, 30-35%, and around 5% for loops, whorls and arches, correspondingly [5]. Furthermore, it is worth noting that these general patterns can be sub-categorized as radial and ulnar loops, plain whorl, central pocket loop, double loop, accidental, as well as plain and tented arches [4].

Fingermarks are typically viewed as the imprints of friction ridge skin on the fingers, deposited on the surface of an object following a touch [8]. In the context of forensic applications, fingermarks are regarded as latent, patent, and plastic [3,7]. Latent fingermarks are frequently discovered at crime scenes [8], and they remain an ongoing problem for forensic investigations due to their readily unseen nature. Consequently, the application of optical, physical, and/or chemical visualization techniques is essential for comparison and identification [8,9]. Patent fingermarks are readily discernible prints that can be transferred onto colored media, such as blood and paint. In contrast, plastic fingermarks are impressions left on soft and malleable surfaces, like putty and wax. The patent and plastic fingermarks are more easily analyzed in forensic investigations when compared with those of latent fingermarks [5,9] (Figure 1).

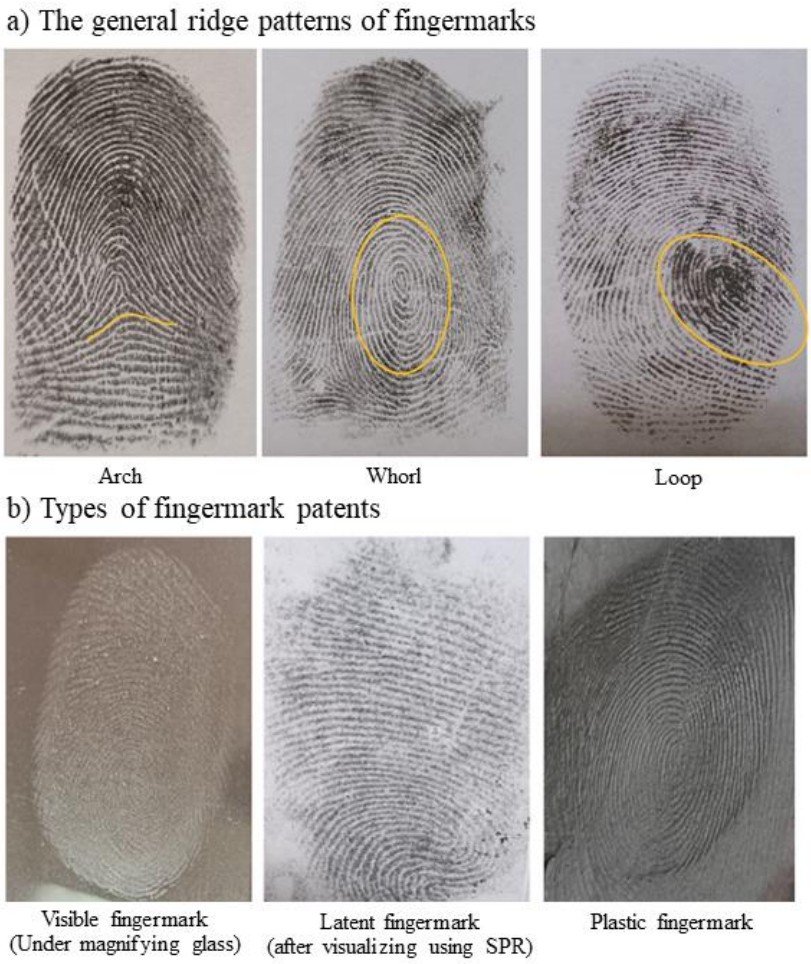

Figure 1. (a) The general ridge patterns and (b) types of fingermark patents.

Conventional visualization technology for wet non-porous objects and its limitation

The choice of visualization techniques is contingent upon the characteristics of the surface under investigation, such as its porosity (porous, semi-porous, or non- porous), levels of moisture (wet or dry), and composition, *viz.* amino and fatty acids [10]. The order in which these techniques are applied also plays a role in decision-making [11]. In the context of forensic casework, latent fingermarks on wet, non-porous objects can be visualized *via* suspension and chemical techniques [12]. Given the suspension techniques for visualizing latent fingermarks on wet, non-porous surfaces, utilization of several variants (black, white, and fluorescence) of Small Particle Reagent (SPR), as well as the other powder suspensions, have been reported [13]. It has been hypothesized that SPR selectively binds to the lipid-soluble components found in fingermarks [14,15], corroborated by Goldstone et al. [16], who observed sub-optimal results in the visualization of latent fingermarks subjected to sea spray aerosol using black and white SPR. This outcome could be attributed to the degradation of sebaceous components caused by exposure to sea spray aerosol.

In the conventional sense, SPR refers to a colloidal dispersion comprising minute particles of molybdenum disulfide (MoS_2) suspended in a combination of water and surfactant [11,17]. In addition to the black variant, the SPR is available in two more color options: white and fluorescent. Tze Lin et al. [14] proposed using fluorescence compounds as a potential contrasting agent for dark or multi-colored surfaces. In a study, Dhall and Kapoor [18] developed a new fluorescent white SPR technique by incorporating rose Bengal dye. They found that when visualizing latent fingermarks obtained from

destructive crime scene simulations, the use of fine suspensions of titanium dioxide TiO_2 outperformed the other two combinations, namely zinc carbonate and zinc oxide. The authors also determined that irrespective of the formulations employed, there was a drop in the quality of visualized fingermarks as the exposure duration to the simulated destructive crime scenes was prolonged. Similarly, the study conducted by Rohatgi and Kapoor [19] revealed that an extended duration of immersion had a subsequent impact on the visual clarity of fingermarks, regardless of the surface types employed, as also indicated by Azman et al. [9]. The newly developed SPR-basic fuschin dye formulation had superior efficacy in detecting latent fingermarks on non-porous surfaces submerged in water for a duration of 45 days, as compared to the SPR-crystal violet formulation. Furthermore, Sodhi and Kaur [20] observed that immersing non-porous objects in water for up to 36 hours had produced visually distinct, well-defined, and highly detailed fingermarks. Nevertheless, as argued by Azman et al. [21], the authors failed to provide explicit details regarding the specific source of water utilized (e.g., tap water, pond water, or drainage water), as well as the environmental conditions in which the experiment was carried out (i.e., indoor or outdoor setting).

It is strongly recommended to take precautionary measures when dealing with SPR, as its contrasting chemicals (MoS_2 and TiO_2), as well as surfactant (sodium tetradecyl sulfate (STS)) and stabilizer (ethylene glycol monomethyl ether (DEGEE)), has been reportedly demonstrating toxic properties [22,23]. In this regard, Racovita [24] reviewed all the pertinent published articles relating to TiO_2 toxicity, especially those of its carcinogenicity, and cautioned that "special care is needed when dispersing the catalytic slurries into the environment, to ensure the proliferation of ecosystems is not impacted long-term by the residual accumulation of this mineral". The observed carcinogenicity phenomenon may be attributed to the heightened synthesis of intracellular reactive oxygen species, as suggested by Gao et al. [25]. Regarding MoS_2, prolonged exposure to this substance has been found to potentially result in chronic respiratory symptoms and irritations of the eyes, nose, skin, and lungs [26]. Interestingly, to elucidate the etiology of diseases that can be associated with the exposure towards molybdenum contaminated water, Chen et al. [27] utilized zebrafish (*Danio rerio*) as a physiological model. The authors concluded that such an exposure would lead to induced oxidative stress and impairment of the osmoregulatory functions, evidenced *via* abnormal activities of antioxidant enzymes (*viz.* superoxide dismutase and catalase) as well as malondialdehyde. The authors further reported inhibition of Na+, K+-ATPase activity in gills and muscles even at sub-chronic exposure. In addition, STS has been classified as a material falling within the category of skin corrosion, specifically subclass 1B (Skin Corr. 1B, H314). Regarding DEGEE, a recent study by Srivastava et al. [28], examined its potential nephrotoxic effects, which were found to vary according to the dosage, duration, and method of administration. In this context, it is essential to acknowledge the potential negative consequences arising from extended exposure to these four hazardous chemicals, particularly their infiltration into aquatic environments and subsequent ecological impact. The rinsing steps required in processing latent fingermarks can be correlated with this phenomenon [9]. Furthermore, despite SPR formulations being routinely used, a thorough examination of existing literature indicates the lack of precise elucidation about the interactions and mechanisms underlying the successful visualization of latent fingermarks through the utilization of SPR compounds. Given these constraints, it would be beneficial to investigate the potential for future exploration of the theory around these interactions.

Relevance of microbial lipases as fingermark biosensors

Considering the human and environmental toxicities relating to the chronic build-ups of TiO_2 (in white SPR) and MoS_2 (in dark SPR) in the aquatic ecosystem, augmented by the presence of surfactants and solubilizers *viz.* STS and DEGEE, researchers have advocated the need to develop greener fingermark visualization reagents [9]. In this regard, the specific attempts made by Azman et al., [9,29] to develop the novel green Nanobio-based reagent (NBR) using *Candida rugosa* lipase (CRL)-multiwall carbon nanotubes (MWCNTs) (CRL-MWCNTs), as a potential fingermark visualization reagent candidate prove forensically and scientifically relevant. However, their results have shown that the NBR favored groomed

fingermarks rather than natural ones, and in several instances, SPR performed better than NBR. The efficacy of the NBR for better visualizing the former was justified by the gas chromatography results, which showed the predominant presence of long-chain fatty acids (*viz.* C16, hexadecanoic and C18, octadecanoic acids) on their fingermark samples that had been long-submerged in water [29]. This poses an issue as CRL is arguably a type of lipase that prefers shorter-chain fatty acids (C4, C8, C10 and C12) [30,31]. Because long chain fatty acids predominate in four-weeks water-submerged fingermarks [29] the efficiency of CRL as a biosensor may be reduced, which explains the lower performance of the NBR than SPR in several instances.

Having considered such a premise of argument, utilization of lipases with higher preferences towards a wider range of fatty acids than that of CRL may prove as a diligent approach for developing a versatile, novel and greener nano-biobased fingermark visualizε(vi) reagent. In this regard, the *Rhizomucor miehei* lipase (RML) may prove to be a good biosensing agent for detecting long-chain fatty acids in extended water-submerged fingermarks based on its broad fatty acid preference, including those between C10- C22 [32]. The RML's strong specificity and substrate versatility make it a suitable biosensing agent to detect the above-said fatty acids, which tests are normally done under ambient conditions. Interestingly, while many positive attributes can be associated with RML in biotechnology, its utilization as a candidate for detecting and visualizing latent fingermarks on non-porous objects submerged in aquatic environments remains unreported in the literature, so far.

Rationale and scope of this review

It is important to indicate here that while various review articles relating to the conventional fingermark visualization technology have been published [9,33,34], specific discussions on the contextual importance of microbial lipases, in view of its applicability, advantages and disadvantages for the same purpose, remains unreported. In this context, having a specific discussion on the issue would unveil the feasibility of the different lipases, what needs to be done to improve their optimum synthesis, sensitivity and contrast of the enzyme nanoconjugates visualized fingermarks. As such, the objective of this review paper is to reposition the contextual aspect of microbial lipases as candidates for producing green fingermark visualization technologies, in compliance with the prevailing guidelines i.e., International Fingerprint Research Group (IFRG) [35].

The scope of this review manuscript includes:

(i) Microbial lipases and its general utilization in biotechnology
(ii) Constituent of the fingermark (water soluble and non-water soluble)
(iii) Utilization of nanobiotechnology for fingermark visualization and related issues
a. Immobilization of CRL onto MWCNTs and its statistical optimization as a means to improve enzyme stability and fingermarks contrast
b. RML-nanoconjugate and its potential as a new fingermark visualization reagent
(iv) IFRG guidelines for developing new fingermark visualization methods/reagents and
(v) Challenges and future insights.

Microbial lipases and its general utilization in biotechnology

Lipases are recognized as the third most extensively utilized enzymes in commercial applications (behind proteases and carbohydrases), covering the share of over 20% in the global enzyme industry [36,37]. Azman et al. [9], indicated that lipases, also known as triacylglycerol acylhydrolases (EC 3.1.1.3), belong to a category of hydrolytic enzymes that facilitate the breakdown of insoluble triacylglycerol into glycerol, acylglycerols, and free fatty acids. Lipases prefer long-chain triacyl-glycerols, which possess limited solubility in aqueous environments. The catalytic reaction of lipases occurs at the interface between lipids and water [38]. Owing to their exceptional stability under extreme temperatures, pH, and organic solvents, lipases have remarkable efficacy in facilitating processes in both aqueous and non-aqueous environments [39]. According to Khan et al. [40], lipases are recognized for possessing a hydrophobic lid, which plays a crucial role in facilitating their interfacial activity. Lipases have been produced by various species of plants, animals, insects, and microbes [41], exhibiting significant variations in their characteristics [42].

Being one of the most versatile biocatalysts in biotechnology, lipases are used in various industries *viz*. food, detergent, pharmaceutical, leather, textile, cosmetic, and biodiesel productions, as well as paper

[43–45]. In particular, lipases from microbes (microbial lipases) have garnered more substantial industrial interests than those of plants and animals, attributable to their favorable characteristics and functional efficacy under extreme conditions, stability in organic solvents, chemo-selectivity and enantio-selectivity, as well as they are independent of co-factors [46,47]. Table 1 represents the different types of microbial lipases as well as their industrial applications. Lipases are classified as serine hydrolases and their enzymatic functions are dependent on a catalytic triad, consisting of Ser-Asp/Glu-His, which is characterized by a consensus sequence (Gly-x-Ser-x-Gly) [36]. The unique α/β hydrolase fold is shown by the three-dimensional structure of lipases [48]. The α/β hydrolase fold enzyme possesses an active region with three catalytic residues: the nucleophilic, catalytic acid, and histidine residues [36].

Lipase production has been documented in various bacterial genera, including *Acinetobacter* [49,50], *Bacillus* [51], *Burkholderia* [52,53], *Pseudomonas* [54], *Staphylococcus aureus* [55], *Microbacterium* [56], *Lactobacillus* [57], *Serratia* [58], *Aeromonas* [59], *Arthrobacter woluwensis* [60] and *Stenotrophomonas maltophilia* [61] among many others. Nevertheless, it is widely acknowledged that the genera *Bacillus* and *Pseudomonas* are the most notable producers of lipases [62]. Bacillus genera, including *Bacillus subtilis, B. licheniformis, B. pumilus, B. alcalophilus, B. coagulans, B. stearothermophilus, Pseudomonas sp., Burkholderia* sp., and *Staphylococcus* sp., are among the most commercially important lipase producers [63–66]. Lipase-producing bacteria have been discovered in several environments, including oil industrial wastes, vegetable oil processing companies, dairy plants, paper industries, and oil-contaminated soil. For instance, while Tripathi et al. [56] discovered eight lipase-producing bacteria in pulp and paper mills, Bharathi et al. [67] reported on the five lipase-producing bacterial strains in petrol-spilled soil.

Moreover, several studies have been conducted in recent decades on the release of lipase from fungal and yeast strains. Several studies have reported the presence of lipases derived from several fungi, including *Aspergillus oryzae* [66], *Mucor circinelloides* [68], *Penicillium* [69], *Rhizopus* [70,71], *Fusarium graminearum* [72], and *Geotrichum candidum* [73,74]. Fungal strains have been identified as promising producers of lipase, possessing distinctive catalytic characteristics that hold significant relevance in diverse commercial applications [75]. The majority of lipase-producing fungi that are of economic and industrial significance can be classified into many genera, including *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., and *Mucor* sp. [75–77]. The lipase production by fungi exhibits variation depending on the strain and content of the growth media, including the carbon and nitrogen sources [75]. Filamentous fungi are recognized as proficient producers of lipase among microbial sources, and the procedures for extracting, purifying, and processing lipases derived from these fungi are comparatively straightforward. In a study [77], the authors had successfully recovered a strain of *Aspergillus aculeatus* from soil contaminated with dairy manure with the lipase generating activity of 9.51 U/mL. In another example, Rahman et al. [78] provided a comprehensive account of the initial immobilization process of *Rhizomucor miehei* lipase (RML) onto ternary blend nanoparticles to enhance the esterification synthesis of pentyl valerate (maximum yield of 97.8%). The process was optimized by the application of response surface methodology (RSM) using a three-level-four-factor Box- Behnken design (BBD).

Lipase production from yeast has distinctive uses within the chemical, medicinal, and biodiesel production sectors [56]. According to a recent literature analysis, it has been found that *Candida utilis* [79], *Candida rugosa* [9,80], *Rhodotorula* sp., [81], *Yarrowia* sp., [82] *Pichia kudriavzevii* [83] and *Pichia pastoris* [84] are considered as the most effective and predominant lipase producers. *Candida* sp. has been identified as the most promising lipase producer among yeasts [85]. Extensive documentations exist regarding the biochemical, structural, and lipase catalytic features derived from *Candida* sp. [86]. In a study, He and Tan [87] investigated the production of the lipase by *Candida* sp. The researchers reported a measured activity of 9,600 U/mL. In a study conducted by Rajendran et al. [88], it was observed that C. *rugosa* had an optimal lipase activity of 3.8 U/mL.

Table 1. The different types of microbial lipases, as well as their reported industrial applications

Microorganisms	Applications	References
	a. Bacteria	
Acinetobacter	Wastewater treatment Soil bioremediation	[49,93]
Bacillus	Food industry (Coconut oil cake)	[51]
Burkholderia	Biodiesel production	[52,53]
Pseudomonas	Leather processing Biodiesel production	[54]
Staphylococcus aureus	Detergent	[55]
Microbacterium	Biodiesel production	[56]
Lactobacillus	Food industry (Flavor esters)	[57]
Serratia	Food industry (Milk isolate)	[94]
Aeromonas	Wastewater treatment	[59]
Arthrobacter woluwensis	Bioconversion of paper mill sludge	[95]
Stenotrophomonas maltophilia	Wastewater treatment	[61]
Bacillus subtilis	Waste cooking oil	[64]
Bacillus licheniformis	Detergent Biodegradation industry	[66]
Bacillus pumilus	Food industry	[96]
Bacillus coagulans	Food industry	[97]
Geobacillus stearothermophilus	Detergent formulation	[65]
Pseudomonas sp.	Biodiesel production	[63]
	b. Fungi	
Aspergillus oryzae	Waste cooking oil soil	[66]
Mucor circinelloides	Renewable substrates for environmental	[68]
Penicillium	Wastewater treatment	[69]
Rhizopus	Oil contaminated soil	[70,71]
Fusarium graminearum	Food industry	[69]
Geotrichum candidum	Biotechnology Bioremediation Detergent	[73,74]
Aspergillus aculeatus	Agricultural industry	[77]
Rhizomucor miehei	Biodiesel Agro-industrial Food Industry Forensic application	[84,98,99]
	c. Yeast	
Candida utilis	Food industry	[79]
Candida rugosa	Biodiesel production Forensic application	[9,80]
Rhodotorula sp.	Biotechnology	[81]
Yarrowia sp.	Environmental pollutant dyes	[82]
Pichia kudriavzevii	Wastewater treatment	[83]
Pichia pastoris	Biodiesel	[84]

The lipase-producing microbes are found in many habitats such as industrial wastes, vegetable oil mill effluent, dairy effluent, oil-contaminated areas, decaying foods, and hot springs [36]. Most microbial lipases are mostly found outside of the cell and are released into the growth media after lipolytic bacteria have utilized the components of the medium. This release occurs in the presence of appropriate inducer substrates and under optimal fermentation conditions [36]. Nevertheless, the production of these biocatalysts differs depending on the careful choice of microbial strains, substrate type, and fermentation method [89]. The production of microbial lipase exhibits temporal variation, ranging from a span of several hours to several days, occurring specifically during the late exponential or stationary growth phase [69]. The biocatalysts are produced using either submerged or solid-state fermentation in various systems, including batch, repeated-batch, fed-batch, or continuous systems [69]. Nevertheless, submerged fermentation, which entails the development of microbes as a suspension in a nutrient-rich broth, is predominantly favored due to its readily controllable process and the substantial quantities of extracellular enzymes released into the growth media [90]. Furthermore, submerged fermentation offers the advantage of achieving a higher level of uniformity in the culture media, facilitating the extraction of lipase from the fermentation medium, and preventing the formation of unwanted metabolites [67]. Approximately 90% of industrial biocatalysts are produced by submerged fermentation [91].

Constituent of the fingermark (water soluble and non-water soluble)

The natural secretions from the fingertips are derived from three distinct types of glands: apocrine, eccrine, and sebaceous. Apocrine glands are located in the axillary regions of the human body, specifically in the armpit and genital regions. The onset of their activity occurs at puberty, and their functioning is regulated by adrenergic neurons [7]. The apocrine sweat has been found to contain proteins, ammonia, carbohydrates, ferric ions, cholesterol, and androgen steroids. The repeated contact of fingertips to the areas of the body containing apocrine sweat glands has the potential to generate latent fingermark residue [7]. Moreover, eccrine glands are extensively dispersed throughout the human body and particularly concentrated on the palmar surfaces of hands and the plantar surfaces of feet [5,7]. The eccrine glands are responsible for the secretion of perspiration, consisting of no more than 20% water [92]. These glands produce various inorganic and organic compounds as a consequence of overall anabolism and catabolism processes [8]. Eccrine sweat is composed of proteins, urea, amino acids, uric acid, lactic acid, sugars, creatinine, and choline, as documented by previous researchers [50]. On the other hand, sebaceous sweat comprises glycerides, fatty acids, wax esters, squalene, and sterol esters [5].

The chemical constituents of sweat residue can be categorized into two main classes: soluble and insoluble in water [8]. The water-soluble constituents include amino acids and inorganic ions (e.g., sodium, potassium, and chloride) [7]. As for the water-insoluble constituents, proteins, lipids, and fats are the major components. The water-insoluble constituents can be further separated into two subcategories: robust and labile [7]. The robust fraction consists of proteins and lipo-proteins, whereas the labile fraction is made up of saturated and unsaturated fatty acids, triglycerides, and lipids. While the labile components experience swift chemical changes when exposed to air, the performance of these components remains unaffected by their exposure to water. On the other hand, robust components establish a strong hydrogen bond with the cellulose composition of paper, resulting in their prolonged retention on its surface [7].

Extensive studies have been conducted on the analysis of amino acids present in fingermark residue, employing various analytical techniques. These approaches include the use of ninhydrin, 1,8-diazafluoren-9-one (DFO), and indanedione [7]. Among these techniques, thin-layer chromatography (TLC) has been employed since early times, while more recent approaches include laser desorption ionization techniques (LDI) or in conjunction with a surface-assisted LDI, coupled with a time-of-flight mass analyzer (TOF) and mass spectrometry (MS) or imaging mass spectrometry (IMS). Various solvent systems, including sodium hydroxide, ethanol, and pyridine, were employed to extract numerous amino acids. These amino acids can be further subjected to derivatization using ethyl chloroformate, before performing instrumental analyses [7]. The interest in amino acids in fingermarks can likely be attributed to

their status as target compounds for commonly employed detection techniques on porous surfaces.

In situations whereby the water-soluble amino acids could have been removed or dissolved by water, the detection of water-insoluble constituents of fingermarks may prove to be a prudent approach for visualization [9,100]. Several substances derived from sebaceous sources were also detected in the residue of fingermarks. Sebum contains components such as glycerides, cholesterol, cholesterol esters, and free fatty acids, primarily originating from the epidermis (hydrolipidic film) [5]. Free fatty acids are the predominant kind of lipid molecules that have been detected in fingermark residue. The fatty acid species that have been identified are octanoic, nonanoic, decanoic, dodecanoic, tridecanoic, myristoleic, myristic, pentadecenoic, pentadecanoic, palmitoleic, palmitic, 9-hexadecenoic, margaric, heptadecenoic, linoleic, oleic, stearic, nonadecanoic, eicosanoic, heneicosanoic, docosanoic, tricosanoic, tetracosanoic acids [101]. Despite the extensive list provided by the previous researchers [101], it is interesting that the lipids/fatty acids for wet fingermarks are scarcely reported in the literature. Specifically, Azman et al. [29] reported that their chromatographic analysis of wet fingermarks only revealed the presence of hexadecenoic and octadecanoic acids as the prevailing lipids on the fingermarks they investigated. Therefore, further studies in this regard appear pertinent to reveal the types of lipids on fingermarks submerged in varying types of water as well as durations. The information may prove useful in performing computational studies to reveal the potential use of such lipids in developing fingermark visualization technology and, subsequently, the laboratory proof of concept.

Utilization of nanobiotechnology for fingermark visualization and related issues

The discipline of biotechnology encompasses a vast array of scientific endeavors, which can be succinctly described as advancing technological applications rooted in biological principles [78]. Biotechnology is a dynamic and interdisciplinary domain that significantly influences various sectors, covering agriculture, veterinary medicine, pharmaceuticals, and the creation of fine chemicals [102]. The technology in question is increasingly being recognized as a prominent solution as it holds significant potential for addressing pressing social issues like safeguarding public health, ensuring an adequate supply of food and energy, and protecting the environment [102–104]. Taniguchi was the first researcher to coin the idea of nanotechnology in 1974, and since then, the topic has attracted a great deal of attention. The term "nano" originates from the Greek language, and it carries the meaning of being little or dwarf-like. In a similar vein, nanoparticles can be delineated as particles that span a size range of 1 to 100 nm, with the potential to extend beyond a few hundred nm. Nanoparticles are, in fact, assemblages of atoms, ions, and molecules, denote a unit of measurement equivalent to one billionth of a meter (10^{-9} m) [78]. As such, nanobiotechnology can also be regarded as the nexus of nanotechnology and biotechnology, which intends to create, improve, and utilize nanoscale structures for advanced biotechnology [102]. In fact, biological agents such as microbes, enzymes, and plant extracts are often coupled with nanoparticles in nanobiotechnology to improve industrial and chemical processes [10].

Given the growing need to visualize latent fingermarks for forensic identification, as well as the potential negative impacts that certain chemicals may exert on both human health and the environment, it is increasingly important to explore more environmentally friendly and safer alternatives for the development of fingermark visualizing reagents [21]. Ideally, these alternatives would be derived from nanobiotechnology methods. However, unlike other fields of study, the utilization of nanobiotechnological approaches for the development of green forensic fingermark visualization technologies has been scarcely reported in the body of literature. The relevant literature on the application of the nanobiotechnological route of fingermark visualization technologies can be grouped into three distinct approaches, as detailed below.

The first approach involves the synthesis of nanocarbon and/or nanosilica particles from agricultural wastes like rice husk [105–107] and bamboo leaves [34] as the by-product of acid digestion, in view of their utilization as green fingermark reagents for visualizing fingermarks on dry non-porous and semi-porous objects. Rajan et al. [105] indicated that the observed clear and sharp images of fingermark ridges prove the selectivity of the nanoparticle towards the fingermark residues, in addition to being easily synthesized from rice husk,

while having low toxicity and energy efficient. The approach is also environmentally friendly since it helps to transform agricultural waste into a beneficial use. In a later study, Rajan et al. [107] successfully synthesized and characterized spherical fluorescent silica nanoparticles from rice husk using curcumin pigment derived from turmeric. The authors reported that the fingermark ridges were enhanced with good contrast and no excessive background interference, arguing that its effectiveness was as good as the commercially available fluorescent fingermark reagent. The same group of researchers [106] further reported the optimized condition for fabricating mono-dispersed spherical silica nanoparticles from rice husk as an attempt to improve the sensitivity and selectivity of the reagent for visualizing latent fingermarks, as well as elucidating the factors affecting the precipitation of silica nanoparticle. Following their successful attempt, the authors accentuated the need to explore the structural changes of the silica nanoparticles when subjected to different neutralization processes in explaining the variations in silica morphology. However, the fact that all the three studies did not comply with the prevailing guideline for the development of a fingermark reagent by the IFRG [35], a specific attempt involving an improved experimental design with a larger sample size may prove necessary in assessing the suitability and applicability of those nanoparticles for routine forensic casework. Moreover, while their methods worked on dry objects, their suitability for visualizing latent fingermarks on non-porous objects submerged in water has not been explored.

The second nanobiotechnological approach discovered in the literature relates to the application of *Candida rugose* lipase (CRL) nanoconjugate, recently coined as the nanobio-based reagent [9,10,14,29,100,108,109]. During their first attempt, Azman et al. [108] synthesized a novel safranin-tinted CRL nanoconjugates reagent that successfully visualized latent fingermarks on non-porous objects submerged for 15 days in a natural outdoor pond, comparable in performance with that of SPR. Nevertheless, the approach seems arduous and time-consuming, requiring three distinct solutions within a nine-minute timeframe for fingermark visualization. Furthermore, it should be noted that the methodology employed in this study [108] did not adhere to the established parameters set forth by the IFRG [4], hence restricting its overall recognition within the forensic fingermark community. In their subsequent attempt to address the limitations, Azman et al. [9] simplified the reagent by factoring out safranin and glutaraldehyde, leaving only CRL immobilized onto MWCNTs) (CRL-MWCNTs that they coined as NBR. Because of the high specificity and selectivity of CRL towards the lipid-soluble components of wet fingermarks, NBR yielded good quality visualized fingermarks over the commercially available conventional SPR (average UC comparison scale of +1). Characterization of the NBR was made using attenuated total reflectance Fourier transform infrared (ATR-FTIR), and field emission scanning electron microscopy (FESEM) was used to confirm the adhesion of NBR onto wet fingermarks. The existence of specific lipid-soluble components (n-hexadecanoic and n-octadecanoic acids) in wet fingermarks was further verified using gas chromatography-mass selective detector (GC-MSD) analysis. These findings were further utilized to propose potential chemical interactions between the NBR material, and the lipid-soluble constituents found in wet fingermarks.

Next, considering the need to optimize the synthesis conditions for NBR, statistical optimization *via* the Box-Behnken Design (BBD) of response surface methodology (RSM) was attempted [10]. The authors reported that the optimal condition (i.e., 100 mg of CRL, 75 mg of acid-functionalized multi-walled carbon nanotubes, and a 5-hour immobilization period) resulted in the highest average quality of visualized fingermarks. This reagent proved to be sensitive enough to detect even faint fingermarks (especially on glass slides), even after a duration of four weeks in storage (both refrigerated and hot conditions), without the use of any preservatives. To replicate a realistic scenario, the optimized NBR was then used (in comparison to the commonly employed SPR) to efficiently visualize latent fingermarks on wet, non-porous surfaces (glass, laminated plastics, and aluminium sheets) that were submerged for four consecutive weeks in an outdoor pond environment. Their results revealed that the NBR was better at discovering fingermarks that had been submerged for a longer period. This finding supported the fact that it could be used for forensic investigations since underwater evidence is usually found long after the crime. The NBR also scored 76 on the greenness test,

which insinuates an "excellent green analysis" [9]. It is important to note that their study was partly in Phase 2 according to the guidelines released by the IFRG [35]. Therefore, the NBR needs to undergo two more evaluation stages (Phases 3 and 4) before it can be commercialized for forensic use.

Ting et al. [100] evaluated the performance of NBR in visualizing latent fingermarks on glass slides submerged in different levels of water salinity for mimicking forensic evidence disposed of in estuaries and swimming pools. Their results revealed that the NBR demonstrated its potential as a more environmentally friendly alternative to SPR in visualizing latent fingermarks on glass slides submerged in varying levels of salinity. However, the fact that the assessment was made in a controlled laboratory condition, the actual ability of the NBR to visualize such fingermarks in high salinity water remains unknown. In another study, Wahab et al. [10] utilized the RSM-optimized potassium triodide-enhanced MWCNTs supported lipase as a potential candidate for green fingermark visualization technology. It was evident that adding potassium triiodide as the mordant expedited the overall staining process. It was found that the mean quality of fingermarks was better for samples immersed for one day than those of 15 days, indicating the effective usage of CRL in the formulation. Therefore, RSM was proven to be dependable in forecasting the optimal condition that resulted in the highest average fingermark quality for both time durations (one and 15 days).

Thirdly, Jiang et al. [110] suggested the application of the assembly of black phosphorus quantum dots-doped metal-organic framework and silver nanoclusters as a versatile enzyme-catalyzed biosensor for solution, flexible substrate, and latent fingermark visual detection of baicalin. The biosensor exhibits notable attributes such as heightened sensitivity, selectivity, and stability in detecting baicalin within actual samples. The method involved the utilization of a flexible substrate to enable the visual detection of baicalin's latent fingermark. This was achieved by directly seeing the dual emissive fluorescence color hues, which the naked eye could discern. This study investigated a straightforward and effective semi-quantitative approach for flexible dual emissive fluorescence visual detection, which can potentially enhance the field of chemo/bio-sensors and analytic techniques. Nonetheless, concerted efforts to address such issues appear necessary given that this relatively new approach was developed without considering the common conditions when fingermark-bearing objects are recovered and the specific need to adhere to the prevailing guideline for fingermark reagent development.

Immobilization of CRL onto MWCNTs and its statistical optimization as a means to improve enzyme stability and fingermarks contrast

A review of the literature reveals that CRL has been the only lipase investigated as the candidate for developing the green forensic fingermark visualization technology on non-porous objects submerged in water, attributable to its exceptional natural affinity and specificity for lipids [108,111]. In addition to the diminished catalytic activity and limited stability of the unbound CRL, the lipase molecules exhibited an off-white appearance. It is crucial to emphasize that the fundamental objective of forensic fingermark visualization is to enhance the differentiation between fingermarks and the surface on which they are deposited [21]. Hence, in its unbound state, the CRL may lack the capacity to offer the essential differentiation required to clearly demarcate the lipid ridges present in latent fingermarks (off-white appearance) despite its notable effectiveness in selectively detecting them as a biosensor.

MWCNTs have extensive applications in various industrial sectors due to their exceptional characteristics, including a substantially large surface area, extremely low weight, chemical inertness, and thermal stability [112]. Furthermore, the efficacy of these functionalized MWCNTs (F-MWCNTs) as nano-supports could be enhanced through surface functionalization (e.g., with acid) [113]. As a result, such an acid functionalization would anchor the CRL to its sidewalls by introducing several polar carboxylate groups [108]. In addition to facilitating forensic identity through the provision of adequate fingermark contrast (as evidenced by its blackish appearance), conjugation with F-MWCNTs unquestionably enhances the stability, activity, and reaction life of the CRL [114], thereby positioning it as the forthcoming cutting-edge, robust biocatalyst. For example, Azman et al. [10] reported significant enhancements in the mean quality of fingermarks when CRL was immobilized onto F- MWCNTs.

The process of immobilizing enzymes onto different nano-supports can be achieved through several methods, such as cross-linking, covalent binding, inclusion/entrapment, and physical adsorption [115]. Physical adsorption is the most straightforward, cost-effective, and reversible method for immobilizing enzymes while maintaining high catalytic activity [116]. The technique described in the literature involves incubating enzymes and a support matrix for an extended duration, facilitating the effective physical binding of enzyme molecules to the support matrix [114]. In addition, it is possible to achieve physical adsorption by interacting ionic forces between enzymes and the support matrix. This can be accomplished by pre-coupling a ligand with specificity for the target enzyme or by conjugating a substance with a binding affinity to the support matrix [117]. The process in question encompasses the participation of hydrogen bonds and hydrophobic interactions [118].

While the utilization of mild forces for the adsorption of CRL onto the matrix may result in the drawback of enzyme leaching [119], it also presents the advantage of enabling the reloading of lipases into the matrix [116]. The said benefit could be valuable in enhancing the adjustability of CRL loading onto the F-MWCNTs, particularly when there is a requirement for increased differentiation to detect the lipid components present in latent fingermarks. Furthermore, Binhayeeding et al. [120] have documented that the immobilization of CRL onto polyhydroxybutyrate particles, achieved by a combination of adsorption and cross-linking techniques, resulted in the lipase retaining 50% of its catalytic activity even after undergoing 14 cycles. It is crucial to emphasize that the immobilization of the CRL onto FMWCNTs enhances its durability and capacity to withstand many freeze-thaw cycles or variations in ambient temperatures prior to its application in forensic contexts.

Due to its advantages, RSM is a widely used statistical method for optimizing chemical and biological processes [121,122]. While the one-variable-at-a-time (OVAT) strategy for fingermark visualization reagent optimization [18,123] has been duly reported, only two articles *viz* [100,109] reported on the application of RSM in forensic fingermark research. As such, the synthesis of the NBR formulation for latent fingermark visualization on wet, non-porous objects involved optimization of the required parameters (amounts of CRL and F-MWCNTs as well as immobilization intervals (using RSM BBD model), as performed by Azman et al. [109] detailed below.

The authors argued that while being costly and time-consuming, the usual OVAT method for optimizing experiments may be erroneous by neglecting the synergistic effects of other parameters during preparation. RSM allows for fewer experimental trials by statistically computing interactions among independent variables, including their mutual interactions (effects of the amount of CRL versus the amount of F-MWCNTs and the amount of F-MWCNTs versus immobilization interval). Their results revealed that the two most effective formulations were: (1) 100 mg CRL and 60 mg F-MWCNTs with a 10-hour immobilization period and (2) 100 mg CRL and 75 mg FMWCNTs with a 5-hour immobilization period. Both formulations yielded comparable qualitative results (UC scale: 0) with a 0% prediction error, validating the BBD model's capacity to anticipate optimal circumstances with dependability and precision [109]. In this context, it is important to indicate that RSM is not the only statistical optimization reported in the literature. Considering that the Taguchi design-assisted optimization for establishing the best immobilization conditions to hyperactivate and stabilize the CRL onto nano-support has reportedly been successful in identifying the best process conditions in several industrial reactions [124], its application for the development of lipase-based nanoconjugates for forensic fingermark technology appears to have a scientific appeal.

RML-nanoconjugate and its potential as a new fingermark visualization reagent

In light of the studies conducted by Azman et al. [9,21,29] it is evident that the NBR preferred groomed fingermarks rather than those that occurred naturally. Furthermore, their results from chromatographic analysis revealed a significant abundance of long-chain fatty acids, specifically hexadecanoic acid (C16) and octadecanoic acid (C18), on the fingermark samples that had undergone prolonged immersion in water [29]. This situation presents a challenge as it may be argued that CRL is a lipase variant that prefers shorter-chain fatty

acids, specifically those with carbon chain lengths of C4, C8, C10, and C12 [30,31]. The prevalence of long-chain fatty acids in fingermarks submerged in water for four weeks [29] may lead to a decrease in the effectiveness of CRL as a biosensor. This could account for the comparatively lower NBR performance in certain cases compared to SPR observable in their studies [9,21,29,109,125]. Considering such a limitation, it is suggested that employing microbial lipases with greater affinity for a broader spectrum of fatty acids than that of CRL could be a sensible strategy for developing a new, innovative, and environmentally friendly nano-biobased reagent for fingermark visualization.

In this context, RML exhibits a potential as a biosensing agent for the detection of long-chain fatty acids in water-submerged fingermarks due to its wide range of fatty acid recognition, encompassing those within the C10-C22 range [32]. The excellent specificity and substrate diversity of the RML render it as a useful biosensing agent for detecting the aforementioned fatty acids, often conducted under ambient settings. It is noteworthy that although RML in biotechnology possesses numerous favorable benefits, its application as a potential method for unveiling hidden fingermarks on non-porous items submerged in aquatic environments has not been documented in the existing scholarly literature. RML is widely accessible commercially, existing in soluble and immobilized states. The enzyme comprises a solitary polypeptide chain comprising 269 amino acid residues. It possesses a molecular weight of 31,600 Da and exhibits an isoelectric point (pI) of 3.8. RML is acidic as a lipase due to its low pI. This characteristic is attributed to the presence of 35 aspartic and glutamic acid residues in the enzyme, which collectively surpass the combined count of 7 and 10 residues for lysine and arginine, respectively [126]. RML exhibits notable activity and stability as a mesophilic lipase, making it a favorable choice for implementation as an industrial biocatalyst, compared to alternative commercially available lipases [127]. The superimposition of RML structure (open and closed confirmations) are illustrated in Figure 2.

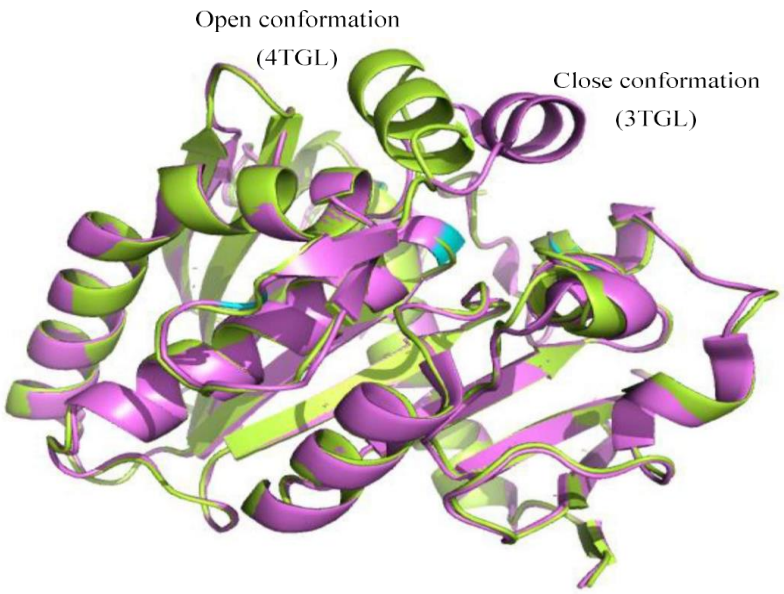

Figure 2. Superimposition of open (yellow, PDB ID: 4TGL) and closed (purple, PDB ID: 3TGL) conformations of the RML using pyMOL software.

RML demonstrated considerable enzymatic activity and stability in low water activity. Consequently, the inclusion of a dehydrating agent in the reaction media is essential to enhance the enzymatic activity of RML to its maximum potential. The tendency of RML to aggregate and be immiscible in aqueous media can be

addressed by incorporating detergent or surfactant compounds, such as sodium cholate, Tween 80, or Tween 20 [78]. These compounds can activate the enzyme, promoting the dispersion of RML in organic media. This activation leads to a significant increase in RML activity, up to three-fold, as demonstrated by Rodrigues and Fernandez-Lafuente [128]. An additional significant aspect in enhancing the interfacial activity of RML involves the utilization of a hinge-type motion of a single helix (residues 83-94). This helix acts as a lid, effectively covering the active site. The introduction of a hydrophobic component or organic solvent is necessary to displace the lid and expose the active site, as this process holds both mechanistic and kinetic significance for RML [129].

IFRG guidelines for developing new fingermark visualization methods/reagents

The guideline for the evaluation of fingermark detection techniques, as outlined by the IFRG [35], was developed through a collaborative process involving the participation of the members, and it received official approval from the IFRG Steering Committee. The scope and purposes as well as the description and requirement for each of the four main research phases, are detailed below. The scope and purpose of the document relate to the effort to provide the "best practice" standards for evaluating new or modified fingermark detection methods/reagents from proving the concept to real application in forensic casework. These standards are not prescriptive, but substantial deviations from them should be clearly noted and explained in presentations and publications. The document also specifies aspects relating to the technology readiness levels, varying scales for assessing the quality of the new or modified fingermark detection process, and key variables (environmental conditions, fingermark composition and age, objects, development and visualization conditions, as well as research implications). Nevertheless, the provided guidance lacks instructions on how to prepare split fingermarks on challenging objects like knives, which is necessary to minimize the inconsistencies within a single donor when employing two different visualization approaches. Given the prevalence of knives as weapons in criminal activities, it is imperative to establish a procedure for preparing split fingermarks on blades to propose innovative or updated visualization methods [21].

Another matter to take into account is the preference of the IFRG [35] for the utilization of natural fingermarks over groomed ones, as stipulated in all four stages of assessment. The relevance of utilizing both natural and groomed fingermarks arises from the observation that offenders while experiencing uneasiness and agitation during the commission of a crime, may perspire and inadvertently come into contact with their faces [9]. This contact can result in the deposition of sebaceous-rich fingermarks [29,89]. Given the potential variations in chemical composition between natural and groomed fingermarks, any proposed revisions to the guideline should consider this element, with a particular focus on the practical applicability of the procedure in real-life crime scenarios.

For every research phase, specifications on the number of donors, number of objects, collection of fingermarks, natural versus groomed fingermarks and standards, as well as quality assessment and reporting procedures, are detailed [35]. Phase 1 study is a pilot study (an initial assessment of a novel fingermark enhancement method). Phase 1 requirements encompass the inclusion of 3-5 donors, with a need for donors representing weak, medium, and strong fingermark donors. Additionally, 1-3 clean objects with low interference should be utilized unless the proposed technique specifically targets difficult objects. Donors should receive instructions on depositing fingermarks, and assistance should be offered as needed. It is preferable to use natural fingermarks and avoid groomed marks whenever possible. Fingermarks should typically be allowed to age for a minimum of 24 hours before development, and the actual age of the fingermarks prior to treatment should be documented and reported. Furthermore, the inclusion of a preliminary performance comparison against relevant routine detection methods *via* suitable comparative scales (e.g., modified-Centre for Applied Science and Technology (m- CAST) and University of Canberra (UC) comparative scales), is necessary.

Bandey and Gibson [130] described that the m-CAST absolute scale categorized the quality of fingermarks into five different grades (0,1,2,3, and 4). While grade '0' refers to no fingermark development, the signs of contact (with less than 1/3 with continuous ridges) is graded as '1'. Whenever 1/3 – 2/3 of the mark with

continuous ridges can be observed, the quality of the fingermarks is graded as '2'; an imperfect mark with more than 2/3 of continuous ridges is considered as grade '3'. Grade 4 refers to the full development of a clear fingermark with continuous ridges [130].

McLaren et al. [131] described a specific scale for comparing the performance of two different fingermark visualization methods known as the UC comparative scale. The assessment of the quality is made based on the scoring system (+2, +1, 0, -1 and -2) detailed below:

(a) +2: half-impression developed by method A exhibits far greater ridge detail and/or contrast than the corresponding half-impression developed by method B.
(b) +1: half-impression developed by method A exhibits slightly greater ridge detail and/or contrast than the corresponding half-impression developed by method B.
(c) 0: no significant difference between the corresponding half-impressions.
(d) -1: half-impression developed by method B exhibits slightly greater ridge detail and/or contrast than the corresponding half-impression developed by method A.
(e) -2: half-impression developed by method B exhibits far greater ridge detail and/or contrast than the corresponding half-impression developed by method A.

When a novel reagent or procedure has been identified as intriguing, it is necessary to subject it to further examination and refinement over a range of experimental circumstances (Phase 2). The objectives of Phase 2 research are (a) to ascertain the optimal reagent formulation, development conditions, and observation parameters, (b) to thoroughly evaluate the strength, responsiveness, and specificity of the novel reagent in comparison to established methodologies and (c) to provide a thoughtful analysis of the performance of the novel reagent when integrated into pre- existing enhancing sequences. As such, Phase 2 research shall involve a sample size of 5-15 donors, preferably with varying concentrations of fingermark secretions achieved using depletion sets. The research shall also include more than 3 typical objects that vary in difficulty or background interference. Donors will be provided with instructions on how to deposit fingermarks, and assistance will be given as needed. Only natural fingermarks will be used unless extreme weather conditions, or the specific focus of the research necessitates otherwise. Fingermarks will be collected and stored for different time periods, depending on the duration of the project and the specific method or scenario under consideration. The quality of fingermark development will be assessed using quantitative absolute and/or comparative assessment scales.

The process of designing a Phase 3 validation project necessitates extensive planning and a strong partnership between research institutions and operational laboratories, particularly when conducted by academic researchers. This collaboration is crucial to guarantee the operational relevance of the validation trials. To effectively evaluate and authenticate the efficacy of a fingermark enhancement procedure, it is imperative to factor in the following experimental characteristics. A minimum of 20 donors, ideally chosen through random selection from the population, should be included in the study. The selection process should involve the processing of anonymous objects that are handled randomly. The number of objects used for the study should be determined based on the project's scope and the specific technique being evaluated. These objects should be representative of typical samples encountered in operational scenarios. The collection of natural fingermarks should be conducted in a blind manner, ensuring that the individuals collecting the fingermarks are unaware of the donor's identity. The samples used in the study should be aged for various periods to simulate different casework scenarios. An absolute assessment scale should be employed to assess the quality of fingermark development. If possible, practitioners with expertise in this field should be involved in the assessment process. Furthermore, the new technique should be evaluated both in terms of its individual performance and performance when used in conjunction with established methods relevant to the study.

Phase 4 initiatives incorporate the refined formulations and development methodologies derived from Phases 2 and 3. In cases where operational laboratories have been engaged in the preceding phases, Phase 4 is frequently regarded as an extension of, or integration with, Phase 3. The conclusive evaluation stage holds significant importance in establishing the suitability of a novel methodology in real-life casework scenarios, hence

determining its compatibility with the laboratory's regular operating procedures. Phase 4 necessitates the evaluation of a novel methodology over a substantial number of cases and potentially across numerous laboratories in the context of national agencies or geographically diverse jurisdictions within a specified trial duration. During this time frame, a comparative analysis is conducted between the efficacy of the novel methodology and the efficacy of existing methodologies. In the case of research conducted in many places, it is important to acknowledge that the laboratory conditions in each setting may vary considerably, potentially exerting an influence on the obtained results. In situations where this may pose a concern, it is advisable to document the temperature and humidity levels within each laboratory and storage facility. When assessing field-based approaches, it is important to document environmental circumstances to ascertain whether they have any influence on the obtained outcomes. The provided material possesses significant value in terms of evaluating and documenting the strength and reliability of a novel methodology. The participating practitioners should evaluate and compare each technique, factoring the average number of usable fingermarks visualized on each exhibit using each technique. If the procedures are arranged sequentially, the experimental method can yield a percentage representing the usable marks created within the sequence. At this point of the research, an absolute scoring system can be implemented to indicate overall fingermark quality, although it is not obligatory.

Challenges and future insights

Chiefly, the utilization of environmentally friendly lipase-based technology for the visualization of latent fingermarks on wet, non-porous objects is a relatively recent development. It is imperative to conduct additional research to identify the specific components that lipases target and quantify their presence. This is crucial because the acceptance of forensic evidence in legal proceedings relies on the technology being widely recognized by the scientific community, as per the Frye standard [5]. As for the Daubert standard, the proposed scientific technology approach must be supported by solid empirical evidence, whereby the role as the gatekeeper is executed by the judge [5]. In addition, it is important to note that the suggested mechanism of interactions between the CRL and fatty acids was derived from the analysis of fatty acids present in fingermarks that were submerged in tap water for 30 days, conducted in a controlled laboratory environment. Furthermore, the previous study [72] suffered from several constraints, including the exclusive utilization of groomed fingermarks obtained from a limited number of donors, i.e., only two individuals for each gender.

Secondly, underwater forensic evidence is typically recovered sometime after the crime/disposal [132], and studies have shown that fatty acid composition changes over time [133], requiring further research into fingermark constituent degradation and aging behavior. Since salt or freshwater may affect fingermark degradation differently, evidence collected in such environments may affect the presence of available/remaining fatty acids. For example, high-salinity water may degrade fingermarks faster than freshwater [134]; therefore, considerable changes in fatty acids and their respective concentrations during a longer period of immersion in water or even in different types of water cannot be excluded. As such, the suggested quantitative study is critical for understanding the substrate preference in aged fingermarks visualizations by lipases, especially on the aging behavior of natural and groomed fingermarks on wet objects.

Next, the NBR developed by the previous researchers only works on white or light-colored objects. Since forensic evidence can be polychromatic, suitable improvements on the NBR to increase fingermark contrast appear necessary. Attaching a fluorescent substance to the NBR would improve fingermark contrast on polychromatic objects recovered from aquatic environments like lakes and rivers. Beyond that, the performance of the NBR in sequence with other relevant fingermark visualization methods has not been thoroughly investigated. This is of tremendous value in forensic investigations, particularly if the NBR can visualize additional fingermarks that cannot be seen using other methods. When the reagent is to be utilized during actual forensic casework, these evaluations are essential in tilting the scales in favor of the NBR.

Subsequently, it is important to emphasize that the CRL prefers for fatty acids with chain lengths of C4, C8, C10, and C12 [86]. Given that the predominant fatty acids

included in fingermarks are often of longer carbon chain lengths, specifically C14-C18 [101], the application of recombinant lipases could potentially yield advantageous outcomes within this particular domain. Engineered custom lipases of this nature might reveal the potential to address the non-reproducibility and inconsistency concerns associated with crude lipases. Moreover, it has been acknowledged that the engineering of recombinant lipases is a more viable approach [135], which warrants further investigation in the context of forensic applications. Lastly, because the NBR developed by Azman et al. [9] appears to be partially at Phase 2 of the four phases prescribed by the IFRG [35] guideline, it must go through two more phases of evaluations (Phases 3 and 4) before the true value of the reagent can be advocated for use in forensic casework applications. Figure 3 depicts the relevance of microbial lipases as candidates for fingermarks visualization reagent, as well as their challenges and future insight in developing environmentally benign fingermark visualization reagents for forensic application.

Conclusion

Fingermarks analysis has long been regarded as a highly significant approach in the field of forensic investigations, serving as a reliable method for human identification. The utilization of SPR for visualizing latent fingermarks on wet, non-porous objects has frequently been proposed for application in laboratory and crime scene settings. Nevertheless, due to the predominant composition of SPR, which consists of carcinogenic and poisonous substances, it is imperative to decrease its regular utilization. In the present setting, it is imperative to develop a sustainable substitute reagent that aligns with the principles of green chemistry. The immobilized CRL has been effectively utilized as a biosensor for lipid constituents in fingermarks, and this progress can be attributed to the implementation of statistically assisted optimization of RSM. Nevertheless, it is important to acknowledge that CRL has several limitations, particularly its limited sensitivity towards long-chain fatty acids. This lack of specificity poses a potential risk to its forensic utility. In this context, employing alternative microbial lipases, such as RML, in the form of nanoconjugates with F-MWCNTs, could offer benefits.

In order to ensure and uphold justice, the admissibility of fingermarks as evidence in a court of law necessitates the analysis of such evidence through methodologies widely acknowledged by the pertinent scientific community. Regrettably, the existing literature lacks sufficient explanations on how visualization reagents interact with the constituents of fingermarks. Given this perspective, it is important to evaluate the scientific and forensic implications while making deliberate efforts to offer information through bioinformatics. Furthermore, the data collected in such studies would serve as a foundation for future advancements in developing cutting-edge green fingermark technology for use in forensic investigations

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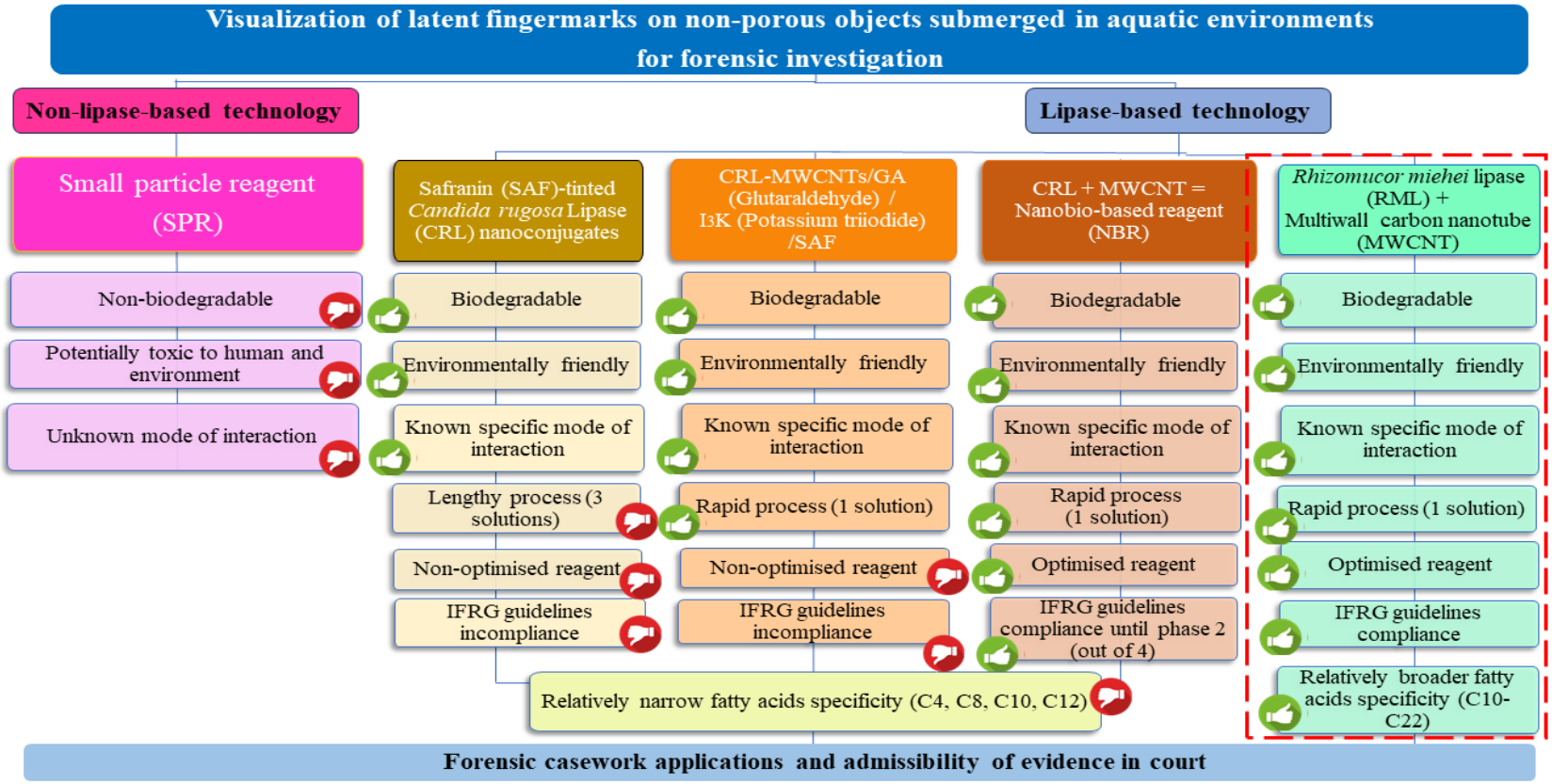

Figure 3. The relevance of microbial lipases as candidates for fingermarks visualization reagent as well as their challenges and future insight.

Red dotted box represents aspects that worth investigation.

Thumb up :refers to positive attributes of methods.

Thumb down : refers to negative attributes of methods.

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