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Protein Localisation in Some Selected Pathogens: A Panacea for Potential Drug Development

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Abstract
Protein localization in pathogens gives information about the virulence of the pathogens. This research is aimed at Z localization of microbial proteins of some selected pathogens using bioinformatics tools. Some isolated soil microbes were identified using conventional methods and 16S ribosomal RNA sequencing. These isolates were found to be homologous to Pseudomonas aeruginosa, Bacillus subtilis, Serratia marcescens, and Bacillus sphaericus (bacterial isolates), while Aspergillus terreus, Candida tropicalis, Penicillium ruben, Yarrowia lipolytica, and Metarrhizium anisopliae. SDS-PAGE was used to separate the proteins according to molecular weight. A. terreus and P. ruben expressed proteins homologous to the molecular weight range. P. ruben and B. sphaericus expressed a glycoprotein of 30kDa. P. ruben and B. subtilis showed protein band homologues to the molecular weight of ribosomal protein S6 (32KDa). P. ruben, M. anisophiliae, Y. lipolytica, B. subtilis, and B. sphaericus expressed spheroplast 40kda, peroxisomal thiolase 42kda, carbonic anhydrase 34.17 kda, ovalbumen 47.05kda, and glutathione transferase 110kda, respectively. All the isolates express cytochrome P450 116kDa. Using bioinformatics tools, Candida tropicalis, Penicillium rubens, Yarrowia lipolytica, and Metarrhizium anisopliae all express wall anchor proteins. Aspergillus terreus, Bacillus sphaericus, and Serratia marcescens express integral membrane proteins that span the transmembrane helix; they are neither exported nor cytoplasmic proteins. Bacillus substilis and Pseudomonas aeruginosa were both predicted to be exported proteins. The location of the various proteins could be related to the survival and virulence of the pathogens. Hence, the knowledge of the protein nature could aid in the development of drugs to target these localized proteins.

Keywords: Protein, Molecular, Weight, Drugs, Bio-Informatics

Introduction

In recent times, the emergence of drug-resistant pathogens has raised the need for new drug development to save mortality and morbidity due to epidemic, endemic, and pandemic threats. *Aspergillus terreus* has been reported to harbor resistance to Amphotericin B antibiotics, causing aspergillosis, a life-threatening disease condition (Blum *et al*., 2008). *Penicillium rubens* is a pathogen associated with critical cases of esophagitis, endophthalmitis, and invasive pulmonary mycosis within immunocompromised individuals (Hupka et al., 2023). Metarhizium anisophiliae has caused sinusitis in immunocompetent hosts (Revankar et al., 1999), *Yarrowia lipolytica* has been reported as a weak human pathogen (Irby et al., 2014), *Candida tropicalis* pathogens have been reported to be one of the most common colonizers of human skin, gastrointestinal tract, and female genitourinary tract (Chai et al., 2010). *Pseudomonas aeruginosa* infection includes malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (Bodey et al., 1983). *Serratia marcescens* causes diseases, including urinary, respiratory, and biliary tract infection, and intravenous catheter-related infections leading to bacteremia (Kim et al., 2015). *Bacillus subtilis* infection includes endocarditis, pneumonia, bacteremia, and septicemia (Tsonis et al., 2018). *Bacillus sphaericus* has also been reported to produce a massive pseudotumor of the lung (Isaacson et al., 1976). Many drugs have a specific target site based on protein interaction in or on the specific localization in the pathogens. Hence, the discovery of new drug targets for pathogenic infections could be of great value for humankind, since this will aid in the control of

rampant drug resistance potential (Ray et al., 2017). The protein localisations provide information on the drug binding site in the target pathogens by enhancing the receptor recognition and cell membrane fusion process (Mittal et al., 2020). Since the drug/pathogen reactions are target specific, the localization of the various proteins' binding sites with respect to different pathogens will give an insight into the drug design model.

Materials And Methods

Isolation and identification of microbes from a soil sample.

Microbial isolation and identification of pathogens from soil samples was carried out via molecular techniques via partial sequences of the 16S rRNA gene. The collected soil was cultured on both general-purpose media and enriched media. Observed growth was isolated and enumerated using the standard procedure (Adamu et al., 2015). The culture medium Nutrient Agar (NA), oxoid of Ijah et al. (2008) was used for the bacteria isolation, while Saboraud Dextrose Agar (SDA) containing 5% chloramphenicol antibiotics was used for fungi isolation.

Cell harvesting and production of clarified lysate

A standard technique adapted from the guide of Ge *et al.* (2018), following some variation, was adopted in the cell collation and lysates of test isolates. The microbial culture harvesting is set up in two stages. At the outset, 25 ml of a 24-hour-old culture was centrifuged for 10 minutes at 5000 x g. The residual cell after the decanted supernatant was stored in a coolant containing an ice bag. The second batch of growth nutrient broth was centrifuged for 10 minutes, at 8000 x g, and the obtained residual cell (pellet) after decanting was washed by re-suspending the pellet via the addition of 40 ml of the spent medium. This was further centrifuged for an additional 10 minutes, at 8000 x g. Lastly, the settled centrifuged pellets were washed buy re-suspending it with 10 ml/g lysis buffer solution (50 mM Tris.HCl, pH 7.9; 0.5 M NaCl). The cells were further disrupted via mechanical thermal shock at consecutive alternation of cells at 43 ºC for 3 minutes and refrigerated temperature. Subsequently, 50 µl of benzamidine and phenylmethylsulfonyl fluoride (PMSF) were introduced to the final mixture, which was further centrifuged for 30 minutes at 24,000 x g in a Sorvall centrifuge with a rotor SS-34 (15,000 rpm) to obtain pellets. The pellet was stored at refrigeration temperature while the supernatant from the lysed cell was loaded on SDS-PAGE for the total protein profile.

Sequence analysis of protein localisation from selected isolates from soil

Nucleotide sequence extraction was collated using PCR machine and conversion of the Nucleotides into peptide sequence was done using bioinformatics tools (Blast and FASTA tools) Protein localization were predicted using bioinformatics tools such as, SignalP-4.1 which indicates peptides with an N-terminal signal which leads the protein through the membrane of the eukaryotic endoplasmic reticulum and across the plasma membrane in prokaryotes, TMHMM (Trans-membrane Hidden Markov Model) 2.0, which predicts the indication of some portion of a protein as embedded in a cellular membrane, and also predicts the path that the amino acid chain follows if the protein is membrane-bound (Chaturvedi *et al*. 2011) Phobius and Tmpred further predicts the transmembrane topology and signal peptides from the amino acid sequence of a protein while the Cwpred tools predicts cell wall proteins (Venko *et al*. 2017)

Results

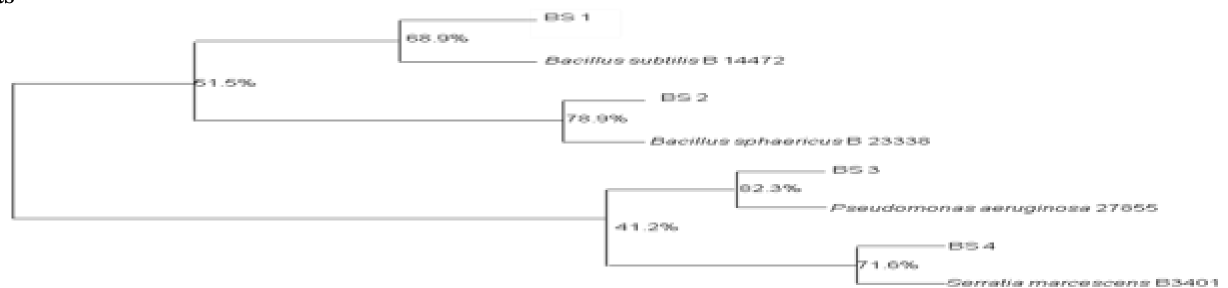

Figure 1: Percentage relative tree of bacteria isolated from soil using the partial sequences of the 16S rRNA gene.

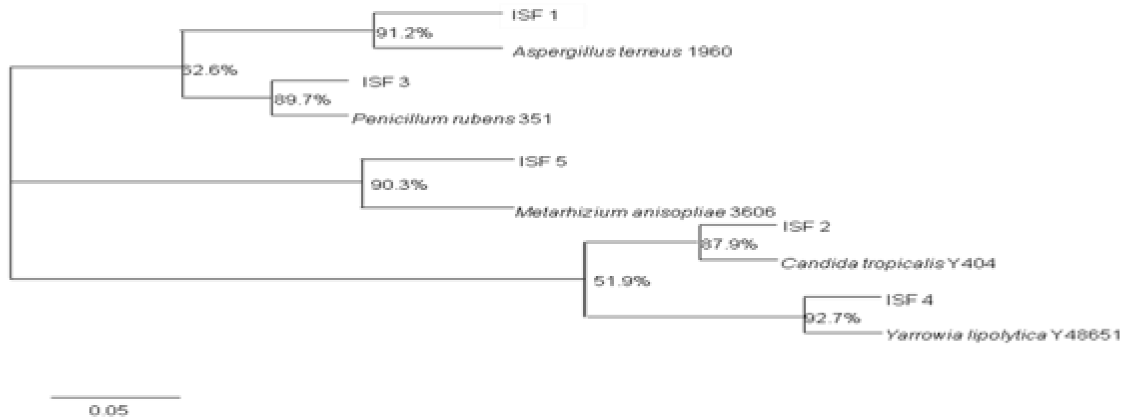

Figure 2: Percentage relativity tree of bacteria isolated from soil using the partial sequences of the 16S rRNA gene.

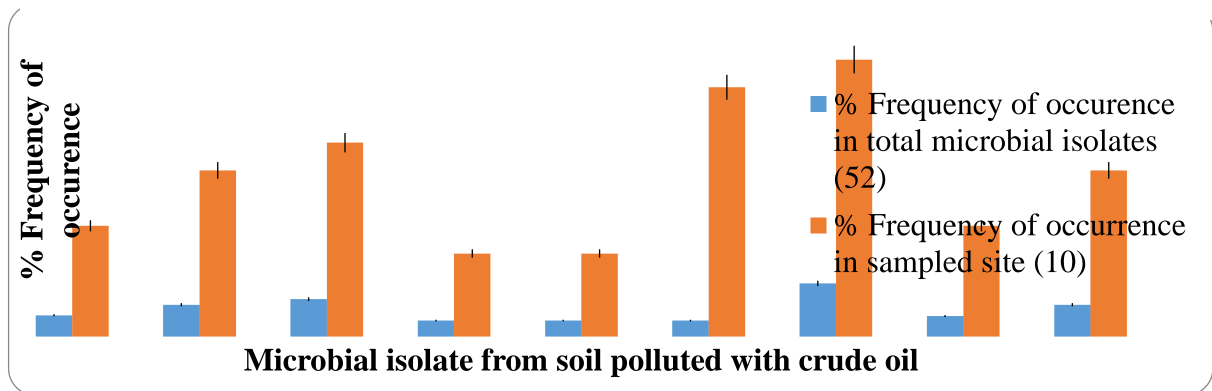

Figure 3: Frequency of occurrence of isolates in polluted soil with crude oil. The average data of two determinations ± Standard deviation (error bar) are shown.

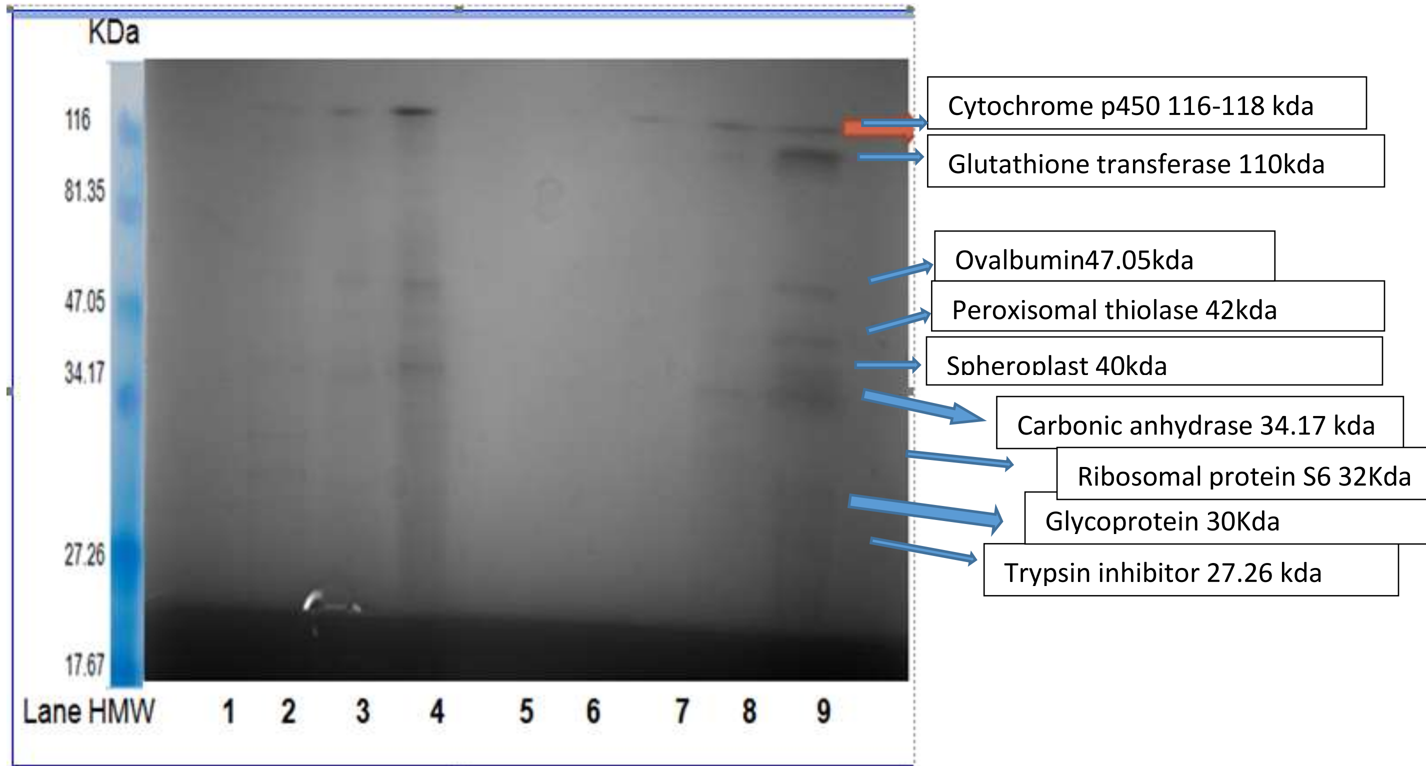

Plate 1: The Sodium Dodecyl Sulphate-Polyacrylamide Gel (SDS-PAGE) of crude membrane protein obtained from selected microbes. **Key**: 1= *A. terreus* 2= *P. ruben,* 3= *M. anisophiliae,* 4= *Y. lipolytica*, 5= *C. tropicalis,* 6= *P. aeruginosa,* 7= *S. marcescens,* 8 = *B. subtilis,* 9 =*B. sphaericus,* HMW =High range Molecular Weights size markers

Table 3: Predicted protein localisation bioinformatics in the selected isolates from soil

SERVER	OUTPUT	AT	CT	PR	YL	MA	BS	BSPH	SM	PA
SignalP v3.0	Prediction	SIGP /NSP	SIGA	NSP	NSP	NSP	NSP	NSP	NSP	NSP
	Probability	0.001	0.011	0.111	0.223	0.000				
	Cleavage site prob.	0.001	0.005	0.105	0.206	0.000				
	Cleavage position	25&26	49&50	26&27	16& 17	28& 29		33& 34	27&28	-1&0
TMHMM v2.0	# of MSD (if any)	0	0	0	0	0	0	0	0	0
	MSD position 1	1-334 OUT	1-269 OUT	1-168 OUT	1-965 OUT		1-431 OUT	1-456 OUT	1-456 OUT	1-488 OUT
	N-in or N-out (only note if MSD present)	N-IN	N-IN	N-IN	N-IN	N-IN	N-IN	N-IN	N-IN	N-IN
Phobius	Is a signal peptide predicted?	NO	NO	NO	NO					
	# of MSD (if any)									
	MSD position 1	1-334 NC P	1-20 CP	1-168 NCP	1-965 NCP	NCP	NCP	1-338 NCP	1-338 NCP	NCP
	2		21-44 TMP					339-356 TM	339-356 TM	
	3		45-269 NCP					357-456 CP	357-456 CP	

TMpred	N-in or N-out # of MSD (if any)	1	1	NO TM	4	1	NO TM	1	1	NO TM
	MSD pos (s) 1	47-67 (21AAs)	126-148 (23AAs)		707-726	122-139		341-357	341-357	
	2				813-835					
	3				878-902					
	4				911-932					
	N-in or N-out		N -IN		N-OUT	N-OUT		N- OUT	N- OUT	N- OUT
PREDLIPO	Verdict e.g.Lipo, Sig, TM,	NO SIGP, TM SEG	NO SIGP	NO SIGP	NO SIGP	NO SIGP, TM SEG				
	Reliability Score	0.744	1.000	1.000	0.987	0.976				
Wall anchor	LPXTG motif in appropriate place identified as wall anchored by CW_PRED	OUT EXTRACELLULAR	1-123 OUT 124-147 TM 148-269 IN	1 OUT 2-21 TM 22-168 IN	1-908 OUT 909-926 TM 927-962 IN	1-120 OUT 121-139 TM 140-169 IN	OUT	OUT	N- OUT	N- OUT
YOUR VERDICT		**IMP**	**WAP**	**WAP**	**WAP**	**WAP**	**EP**	**IMP**	**IMP**	**EP**

N.B. use abbreviations where appropriate e.g. SP, signal peptide; MSD "membrane-spanning domain" = transmembrane helix" TM", CP and NCP "Cytoplasmic protein and Non Cytoplasmic Protein" EP "Exported Protein" WAP "Wall Anchor Protein", IMP "Integral Membrane Protein" Sig A "Signal Anchor**"** AT *"Aspergillius terreus",* CT *"Candida tropicalis",* PR *" Penicillium ruben",* YL *"Yarrowia lipolytica",* MA *"M anisopliae",* BS *"Bacillus subtilis",* BSPH *"Bacillus sphaericus",* SM *"Serratia marcescens",* PA *"Pseudomonas aeruginosa"*

Discussion

A total of four bacterial strains and five fungal strains were selected from soil samples polluted with crude oil on nutrient agar and Sabouraud dextrose agar, respectively. Molecular identification of bacterial isolates (BS1, BS2 BS3 and BS4) and fungal isolate (ISF1, ISF2, ISF3, ISF4 and ISF5) confirmed the isolates to be homologous to *Pseudomonas aeruginosa*, (PA) *Bacillus subtilis,* (BS) *Serratia marcescens* (SM) and *Bacillus sphaericus* (BSPH) (bacterial isolates) while *Aspergillus terreus* (AT), *Candidia tropicalis*,(CT), *Penicillium ruben*,(PR) *Yarrowia lipolytica* (YL) and *Metarhizium anisopliae* (MA) (fungal isolates) in Figure 1 and Figure 2 respectively. Figure 3 illustrates the frequencies of the occurrence of microbial isolates and the frequency of occurrences across the 10 randomly selected sample sites. It illustrated the frequency of total microbial isolates and the frequency of occurrence in ten sample sites from the soil. The microbial isolates and frequency MA (5.8/30) %, PA (17.3/90) %, BS (19.2/ 100) %, SM (7.4/40) %, and BSPH (11.5/ 60) respectively. Plate 1 illustrated the various numbers and molecular weight bands of crude protein extract associated with all the selected isolates from soil. These molecular weights, analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), illustrated several bands ranging from 20.492 to 116 (kDa). The bands on lanes 1, 2, 3, 4, 5, 6, 7, 8, and 9 illustrated correspondence bands from crude protein extract from membrane-lysed isolated microbes of *AT, PR., MA, YL, CT, PA, SM, BS,* and *BSPH,* respectively. AT and PR showed a band close to 27.26 kDa, which is homologous to the Trypsin inhibitor molecular weight range. Glycoprotein (30kDa) reported by Huynh *et al*. (1996) to demonstrate anti-fungal properties against

plant pathogens was expressed by *PR* and *BSPH,* a characteristic reported by Requena *et al*. (2023) and Naureen et al. (2017) for the respective microorganism, hence can serve as bio-control. Ribosomal protein S6 (32Kda) related to 40S subunit ribosomal protein, which plays an important role in the induced cell cycle arrest due to hyperphosphorylation of the S6 and thus correlates to oncogenic Ras –induced cell cycle in activated Xenopus egg extract (Pian et al., 2004), was expressed by *PR* and *BS.* Spheroplast 40kda, Peroxisomal thiolase 42kda, Carbonic anhydrase 34.17 kda, Ovalbumen 47.05kda, and Glutathione transferase 110kda were expressed by *PR, MA, YL, BS, and B.* Carbonic anhydrase 34.17 kDa, which aids in the catalysis of the interconversion of CO_2 and bicarbonate at a rate about 10^7 times more rapid than the uncatalyzed rate. They have been observed to be located in the cytosol and in the extracellular fluid. (Nocentini and Supuran, 2019). Spheroplast 40kda, a protein formed when there is inhibition of cell wall formation due to antibiotics from penicillin and lysozyme, and is further used by the microorganism for enlargement due to a lack of cell wall

Peroxisomal thiolase 42kDa has been reported to have oxidative properties and has also been studied to catalyze the carbon-carbon bond, which aids the biosynthesis pathways for fatty acids and polyketides (Haapalainnen et al., 2006). Ovalbumen 47.05kDa has been associated with biological activities like anti-cancer, anti-hypertensive, antimicrobial, antioxidant, and immunomodulating activities (Zhnag, 2017). Glutathione transferase 110kDa has also been studied to be implicated in the protection of cellular macromolecules from attacks by reactive electrophiles (Townsend and Tew, 2003). All the isolates express cytochrome p450 116kDa, a group of monooxygenase enzymes that play a crucial role in the reclamation of xenobiotics and homeostasis (Nagini, 2018). Extracellular secretion and digestion of most fungi justify the predicted protein localization of *Candida tropicalis*, *Penicillium rubens*, *Yarrowia lipolytica,* and *Metarrhizium anisopliae* as wall anchor proteins. *Aspergillus terreus*. *Bacillus sphaericus* and *Serratia marcescens* express integral membrane protein that span the transmembrane helix, hence they are neither exported nor cytoplasmic proteins. *Bacillus substilis* and *Pseudomonas aeruginosa* were both predicted to be exported proteins. Microbial proteins, as a natural product, have attracted consideration and interest from pharmaceutical and biotechnology corporations as a valuable basis of prospective drug targets (Atanasov et al., 2021). The location of these proteins may determine the pathogenicity and virulence, likewise serving as the receptor and binding site for drugs.

Conclusion

Microbial proteins offer the acute association between genes and disease, hence providing the blueprint that aids in the explanation and comprehension of elementary biological processes ranging from disease pathology, through diagnosis, to treatment. Development of drugs to target these localized proteins could prove promising in the control of the drug resistance threat to human existence.

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