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Full Length Research Paper

Biological screening of microbes isolated from soil of ex-tin mining land in Kampar Area

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Soil samples from ex-tin mining area located in proximity to Universiti Tunku Abdul Rahman (UTAR), Kampar campus was collected and cultured on nutrient agar plates. The morphological characteristics including basic staining, Gram staining and endospore staining of five selected isolates (SL1-5) were studied. All isolates had circular shape, opaque appearance and smooth surface. SL1, SL2 and SL3 were Gram-positive bacilli and endospore formers. SL4 and SL5 were Gram-negative cocci. The sensitivity test towards 17 types of antibiotics was carried out using the disc diffusion method. SL2, SL3 and SL4 were multidrug-resistant isolates. Prominently, SL4 was resistant to 10 types of antibiotics. Antibacterial activity against 11 types of bacteria was also evaluated. SL1 was found to produce antibacterial agent that inhibit the growth of *Salmonella* sp. and *Staphylococcus epidermidis*. Protease, α-amylase, lipase and another 19 types of enzyme activity were screened using enzymatic assay and API ZYM kit assay. All isolates had proteolytic and lipolytic activities. SL1, SL2 and SL5 were able to produce protease enzyme whereas SL4 was able to produce phospholipase enzyme. In API ZYM assay, all isolates were able to produce esterase (C4), esterase lipase (C8) and leucine arylamidase (Leu). However, α-amylase producer was absent. Further studies will be carried out to identify the species of all isolates.

Key words: Nutrient agar (NA), multidrug-resistant, disc diffusion, antibacterial assay, enzymatic assay.

INTRODUCTION

Environmental microbes are capable of producing extra-cellular enzymes and serve good sources of antibacterial substances (Fariha et al., 2009). Soil, a part of the lithosphere is a dynamic ecosystem that supports com-plex interactions between numerous geologic, chemical and biological factors (Talaro, 2009). This region teems with microbes play a very important role in maintaining health of soil, ecosystem functions and crop productivity (Vijendra and Ashok, 2009).

To date, increase prevalence of antibiotic resistance is one of the major concerns in medical research. The inappropriate use of antibiotics especially in human medicine provides selective pressure, which resulted in emergence of antibiotic resistance bacteria and resistance genes. Resistance pathogens have been used by medical chemists as targets to test modifications of existing antibiotics for expanded-spectrum molecules that have regained potency against the resistance microbes (Christopher, 2003). In present study, isolates obtained from soil of ex-tin mining lake were tested on their sen-sitivity towards antibiotics using the Kirby-Bauer method. This antibiotic assay allows rapid determination of drug efficacy and also helps to combat microbial resistance, which enables effective treatment of diseases (James and Natalie, 2008).

Farah et al. (2009) had drawn attention on the isolation and characterization of bacteriocins from indigenous soil

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Abbreviations: NA, Nutrient agar; **P10,** penicillin; **AMP10,** ampicillin; **VA30,** vancomycin; **B10,** bacitracin; **E15,** erythromycin; **C30,** chloramphenicol; **F300,** nitrofurantoin; **AK30,** amikacin; **K30,** kanamycin; **N10,** neomycin; **S10,** streptomycin; **CN10,** gentamycin; **TE30**, tetracycline; **NA30,** nalidixic acid; **NOR100,** norfloxacin, **RL100,** sulphanethoxazole; **W5,** trimethoprim.

associated bacteria. Bacteriocin was important in medical industry and it was a potential food bio-preservative (Fariha et al., 2009). Jos et al. (2002) isolated numerous bacterial strains, which produced antibiotics *in vitro* from different soil and plant host in their studies. The wide distribution of these bacterial strains suggests that the antibiotic-producing bacteria are common constituents of the indigenous microflora in soil. In conjunction with this, the isolates were screened on their antibacterial activity on 11 types of indicator bacteria by using the agar well diffusion method.

The research that carried out by Tambekar et al. (2009) obtained 33% of multiple enzyme producers, which belong to the *Bacillus* species from soil sample. Hence, protease, phospholipase and α-amylase assays were conducted in this study on the attempt to obtain enzyme producers, which might be applicable for various industry purposes. Protease is being used in a wide variety of applications such as detergent industry, leather processing industry, dairy and food industry, protein hydrolysis, peptide synthesis and texture industry (Magdi et al., 2009). Magdi et al. (2009) had studied the production of protease by *Bacillus subtilis* KO strain. Phospholipases are used in industry on degumming of vegetable oils, baking or egg yolk treatment (Maria et al., 2007). Palvannan and Boopathy (2005) had demon-strated that phophatidylinositol-specific phospholipase C was produced by *Bacillus thuringiensis serovar Kurstaki* using potato-based media. α-amylase constitutes approximately 25% of the enzyme market, which cover many industrial processes such as food, textile and paper industry (Tambekar et al., 2009). API ZYM was performed to determine the activity of isolates producing various extracellular enzymes (Mudryk and Podgorska, 2006).

The present study attempted to isolate novel micro-organisms from an ex-tin mining land and to study the morphological and biochemical characteristics of soil microbes from this location. The isolates were screened on their antibiotic sensitivity, antibacterial activity and enzymatic activity, which in future might have potential industrial applications.

MATERIALS AND METHODS

Source of sample

Soil sample was collected from an ex-tin mining area located approximately 1 km from UTAR, Perak campus. The soil sample was then preserved in phosphate buffer solution (PBS).

Isolation

The soil sample was serially diluted with PBS to obtain $1:10^6$. The diluted sample was spreaded on NA surface according to the spread plate method described by Talaro (2009). The plates were then incubated at 30°C overnight. Five isolates were isolated and selected based on streak plate method (Talaro, 2009).

Morphological and biochemical examination of the respective isolates

The morphology of the isolates were observed and recorded. The cell shape and arrangement were determined using the standard procedures of basic stain, Gram stain and endospore stain (Robert et al., 2009). All isolates were screened for the presence of protease, α-amylase and phospholipase on the skim milk agar plates, starch agar plates and egg yolk agar plates respectively as described by Tambekar et al. (2009). The API ZYM assay was also carried out according to the procedure described by BioMeriux SA. The API ZYM stripes were incubated for four hours and results were revealed by the intensity of color formation.

Determination of antibiotic susceptibility (Kirby-Bauer Method)

Susceptibility of the isolates to 17 types of antibiotics was performed by the disc diffusion method as described by James and Natalie (2008). Commercially available antibiotics disc (Oxoid, UK) containing penicillin (P10), ampicillin (AMP10), vancomycin (VA30), bacitracin (B10), erythromycin (E15), chloramphenicol (C30), nitrofurantion (F300), amikacin (AK30), kanamycin (K30), neomycin (N10), streptomycin (S10), gentamycin (CN10), tetracycline (TE30), nalidixic acid (NA30), norfloxacin (NOR100), sulphanethoxazole (RL100) and trimethoprim (W5) were placed on the surface of the agar plates and incubated at 30°C for 24 h.

Inhibition zone diameters were measured inclusive of the diameter of the discs accordingly to the results expressed susceptible/sensitive (≥ 21 mm); intermediate (16-20 mm) and resistant (≤15 mm) by Liasi et al. (2009). *E. coli* (control 1) and *B. subtilis* (control 2).

Screening for antibacterial agent

All isolates were screened for antibacterial activity against 11 indicator bacteria using the agar well diffusion method as described by Ahmed et al. (2008). The indicator bacteria included seven Gram-positive bacteria (*Staphylococcus aureus*, *B. subtilis*, *Bacillus cereus*, *S. epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis* and *Bacillus sphaericus*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella sp.*). The plates were incubated at 30°C for 24 h. Results were expressed as moderate (6-9 mm); strong (10-14 mm) or very strong (15-18 mm) according to Liasi et al. (2009).

RESULTS AND DISCUSSION

From the morphological examination, all isolates had circular shape, opaque appearance and smooth surfaces. Isolates SL1, SL2 and SL3 were Gram-positive bacilli whereas SL4 and SL5 were Gram-negative cocci. All Gram-positive bacilli were spore formers (Table 1 and Figure 1).

The respective isolates were tested on their susceptibility towards 17 types of antibiotics. Antibiotic sensitivity was shown by formation of inhibition zone surrounding each isolate (Figure 2). The degree of susceptibility was measured as diameter of inhibition zone and tabulated in Table 2. Isolates SL2, SL3 and SL4 were resistant to multiple antibiotics with no inhibition zone formed surrounding each isolate. Among the isolates, SL4 had

Table 1. Morphological characteristic of isolate SL1-5.

Isolate	SL1	SL2	SL3	SL4	SL5
Form of colony	Circular	Circular	Circular	Circular	Circular
Translucency and opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation of colony	Convex	Flat	Convex	Flat	Flat
Margin of colony	Curled	Undulate	Entire	Entire	Entire
Surface of colony	Smooth	Smooth	Smooth	Smooth	Smooth
Pigmentation	Light yellow	White	White	White	Orange
Cell morphology	Bacilli	Bacilli	Bacilli	Cocci	Cocci
Gram stain reaction	+	+	+	-	-
Spore stain	Yes	Yes	Yes	No	No
Growth at 30°C	Yes	Yes	Yes	Yes	Yes

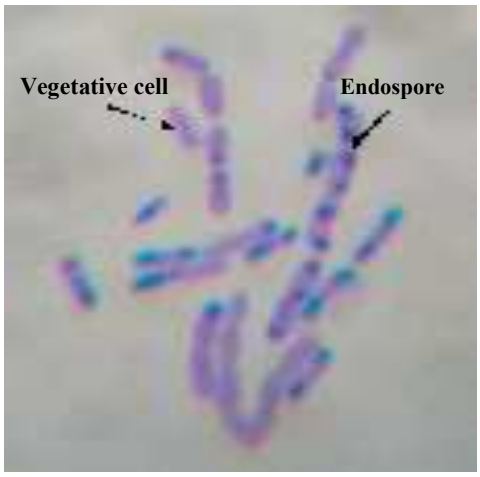

Figure 1. SL2 stained with endospore stain. The image was captured under light microscope with a total magnification of 1000×.

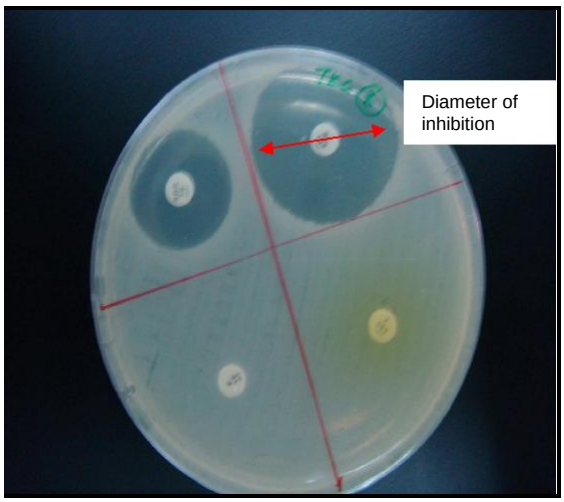

Figure 2. The measurement of antibiotic inhibition zone on Mueller-Hinton agar.

the highest resistance that resistant to 10 types of antibiotics. However, isolate SL1 was susceptible towards 12 types of antibiotics whereas isolate SL5 was susceptible towards 15 types of the antibiotics. 100% of the isolates were sensitive towards tetracycline (TE30). None of the isolates displayed sensitivity towards streptomycin (S10) and vancomycin (VA30).

In present study, tetracycline was the strongest acting group of antibiotics with 100% of susceptibility. Tetracyclines acted to obstruct protein synthesis via binding to bacterial ribosome 30S. Besides that, norfloxacin and sulphamethoxazole were also effective against isolates where 80% of the isolates were sensitive towards both antibiotics. The activity of norfloxacin involves the disturbance of the bacterial DNA replication as a result of impeding the activity of DNA gyrase while sulphamethoxazole susceptibility might due to inhibition of folic acid synthesis by antibiotic competing with p-aminobenzoic acid (Willey et al., 2008). Streptomycin proved to have narrow spectrum of activity against all isolates with 80% of the isolates displayed streptomycin resistance and only 20% of the isolates intermediate to this antibiotic. The mechanism of the streptomycin is similarly to that of tetracycline, which has effects on the 30S ribosome (Willey et al., 2008).

The multidrug-resistance capability of SL2, SL3 and SL4 might be associated with the reduced penetration of the antibiotic into the cell or resulted from active processes such as the change in the transport of compounds through the bacterial cells (Walczak and Donderski, 2004). The resistance isolates in this study might also due to presence of R plasmid in the cells of the isolates tested where a single plasmid may carry gene resistance to several drugs such as aminoglycosides, chloramphenicol, penicillin and others (Willey et al., 2008). R Plasmid can transfer the genetic material among different species through the conjugation and transformation processes. R plasmid plays a vital role in antibiotic resistance where antibiotic-fighting genes are assembled which protect microorganism from being affected via inactivation, creation of substituted

Table 2. Assessment of isolates SL1-5 to 17 different types of antibiotics.

Numbers indicate the diameter of inhibition zone (mm)							
Isolate	**SL 1**	**SL 2**	**SL 3**	**SL 4**	**SL 5**	**Control (*E. coli*)**	**Control (*B. subtilis*)**
Penicillin G					(41)		
(P10)	+++	+++	+++	+++	+	+++	+++
Ampicillin		(11)	(9)	(10)	(42)	(15)	
(AMP10)	+++	+++	+++	+++	+	+++	+++
Vancomycin	(15)	(8)	(8)		(15)	(9)	(30)
(VA30)	+++	+++	+++	+++	+++	+++	+
Bacitracin			(7)		(25)		
(B10)	+++	+++	+++	+++	+	+++	+++
Erythromycin	(22)	(17)	(19)		(12)	(15)	(12)
(E15)	+	++	++	+++	+++	+++	+++
Chloramphenol	(35)				(31)	(32)	(30)
(C30)	+	+++	+++	+++	+	+	+
Nitrofurantoin	(23)				(22)	(42)	(18)
(F300)	+	+++	+++	+++	+	+	++
Amikacin	(22)	(21)	(20)	(20)	(26)	(30)	(21)
(AK30)	+	+	++	++	+	+	+
Kanamycin	(18)	(22)	(21)	(20)	(25)	(32)	(21)
(K30)	++	+	+	++	+	+	+
Neomycin	(16)	(14)	(15)	(19)	(16)	(22)	(11)
(N10)	++	+++	+++	++	++	+	+++
Streptomycin	(15)	(9)	(8)		(20)	(23)	(12)
(S10)	+++	+++	+++	+++	++	+	+++
Gentamicin	(20)	(18)	(20)	(19)	(21)	(29)	(21)
(CN10)	++	++	++	++	+	+	+
Tetracycline	(27)	(29)	(25)	(21)	(30)	(38)	(23)
(TE30)	+	+	+	+	+	+	+
Nalidixic acid	(360	(27)	(24)	(20)	(20)	(31)	(24)
(NA30)	+	+	+	++	++	+++	+
Norfloxacin	(40)	(23)	(23)	(20)	(21)	(42)	(25)
(NOR10)	+	+	+	++	+	+	+
Sulphamethoxazole	(30)	(30)	(33)	(10)	(30)	(43)	(22)
(RL100)	+	+	+	+++	+	+	+
Trimethoprim	(35)	(31)	(12)		(40)	(44)	(23)
(W5)	+	+	+++	+++	+	+	+

Degree of susceptibility: + = susceptible/sensitive (≥ 21mm); ++ = intermediate (16-20 mm); +++ = resistant (≤15 mm).

metabolic paths, impermeability of cytoplasmic membranes as well as alteration at the target site (Mudryk, 2002; Walczak and Donderski, 2004).

In this study, all Gram-positive isolates were resistant against vancomycin. Vancomycin act by inhibiting the cell wall synthesis (Christopher, 2003). Thus resistance to vancomycin in Gram-positive isolates might result from the changing terminal D-alanine-D-alanine in their peptidoglycan to a D-alanine-D-lactate which drastically reduces antibiotic binding. Moreover, high proportion of isolates resistant to the β-lactam antibiotics such as penicillin and ampicillin might due to the capability of those isolates to detoxify antimicrobial agents probably by synthesising three extracellular enzymes named β-lactamase, acylase and penicillinase. On the other hand, high percentage of isolates resistant against streptomycin probably due to the narrow spectrum of activity possessed by this antibiotic which target only on aerobic Gram-negative bacteria (Willey et al., 2008). Moreover, the result also found that, there were 80% of the isolates resistant to bacitracin which is bactericidal against Gram-positive cocci and bacilli, particularly against certain strains of *Clostridia*. It is a polypeptide antibiotic which interferes with bacterial cell wall biosynthesis by inhibiting dephosphorylation of C55-isoprenyl pyrophosphate (IPP) that required as carrier for synthesis of peptidoglycan.

Table 3. Inhibitory spectrum of antibacterial-producing isolates on Gram-positive and Gram-negative Bacteria.

Indicator species	SL 1	SL 2	SL 3	SL 4	SL 5
Gram-positive					
B. subtilis	ND	ND	ND	ND	ND
B. cereus	ND	ND	ND	ND	ND
B. sphericus	ND	ND	ND	ND	ND
M. luteus	ND	ND	ND	ND	ND
S. aureus	ND	ND	ND	ND	ND
S. epidermidis	+ (6)	ND	ND	ND	ND
E. faecalis	ND	ND	ND	ND	ND
Gram-negative					
E. coli	ND	ND	ND	ND	ND
Salmonella	+ (6)	ND	ND	ND	ND
P. aeruginosa	ND	ND	ND	ND	ND
P. vulgaris	ND	ND	ND	ND	ND

Degree of inhibition: + = moderate inhibition zone (6-9 mm); ++ = strong inhibition zone (10-14mm); +++ = very strong inhibition zone (15-18mm); ND = Non-detectable.

Resistance to this antibiotic might resulted from increased *de novo* synthesis of C55-isoprenyl phosphates (IP) (Pollock et al., 1994). Cain et al. (1993) noted that increased intracellular levels of a lipid kinase which is the product of the bacA gene of *E. coli*, confers resistance to bacitracin whereby the phosphorylation of isoprenyl alcohol by the kinase appears to increase the level of the carrier isoprenyl phosphates thereby circumventing the sequestration of IPP by bacitracin.

In term of antibacterial assay, positive result was shown by capability of isolate SL1 to produce antibacterial agent against both *Salmonella* sp. and *S. epidermidis* (Table 3). Based on the morphological study, SL1 might be the member of genus *Bacillus*. According to Dohren (1995), the members of the genus *Bacillus* are important in their antibacterial activity since they produce a variety of peptide antibiotics. The bacitracin, polymyxin, gramicidin, tyrocidine, subtilin and bacilysin are peptide antibiotics of medical importance in the pharmaceutical industry produced by the *Bacillus* species while the bacitracin A is the dominant commercial product (Schallmey et al., 2004). Most of these antibiotics are low molecular weight peptides which are produced via the non-ribosomal biosynthetic pathway with the aid of specific enzymes called peptide synthetases.

Generally, antibiotics produced by *Bacillus* at the early stages of sporulation process (Fariha et al., 2009). The production of bacteriocins or bacteriocin-like substances has been described for *B. coagulans, B. brevis, B. licheniformis, B. cereus, B. subtilis, B. amyloliquefaciens* and other *Bacillus* species (Farah et al., 2009). Inhibition of various organisms had been reported by different scientists. *B. licheniformis* strain 189 was isolated from a hot spring environment in Azores Portugal, which greatly inhibit the growth of Gram-positive bacteria by producing peptide antibiotic (Mendo et al., 2004). Fariha et al. (2009) noted that antimicrobial capabilities of *Bacillus pumilus* SAF1 isolated from soil was tested in both free and immobilized environments and the optimization of different culture conditions for maximum production was determined.

Table 4 showed the assessment of enzymatic activity. Isolates SL1, SL2 and SL5 demonstrated protease enzyme activity with formation of clear zone. SL4 produced phospholipase enzyme with the formation of white opaque zones of precipitate. However, none of the isolates was able to produce α-amylase enzyme. Enzymatic assay was further carried out using API-ZYM kit to screen for the presence of 19 different types of enzymes (Table 5). Interestingly, all isolates produced esterase (C4), esterase lipase (C8) and leucine arylamidase (Leu). However, none of the isolates were able to produce enzyme β-glucuronidase (β-GI) and α-mannosidase (α-Ma).

From enzymatic assay, the esterase, esterase lipase, and leucine arylamidase were found in all the isolates tested. Whereas, the sole protease enzyme producers were isolates SL1, SL2 and SL5 that constituted 60% of the isolates and the SL1 and SL2 might belong to the genus *Bacillus*. Various studies have been carried out to elucidate the extracellular protease enzyme activity of *Bacillus* species from soil. For instance, Folasade et al. (2005) demonstrated that *Bacillus* species exhibited extracellular protease enzyme production activities. Ellaiah et al. (2002) indicated that *Bacillus* species are specific producers of extracellular proteases. Extracellular proteases hydrolyse proteins into mono- or oligomers, mainly peptides and amino acids. Those low

Table 4. Assessment of enzymatic assay.

Enzymes Isolate	Protease	Phospholipase	α-amylase
SL1	++ (8)	ND	ND
SL2	+++ (11)	ND	ND
SL3	ND	ND	ND
SL4	ND	++ (8)	ND
SL5	+++ (12)	ND	ND
Control (*E. coli*)	ND	ND	ND
Control (*B. subtilis*)	++ (5)	+++ (15)	+++ (10)

Degree of enzymatic activity: + = low (≤2 mm); ++ = moderate (>2-8 mm); +++ = high (>8 mm); ND = Non-detectable

Table 5. API-ZYM kit.

Enzyme \ Isolate	SL1	SL2	SL3	SL4	SL5
Control	**0 (-)**	**0 (-)**	**0 (-)**	**0 (-)**	**0 (-)**
Alkaline phosphatase	5 (+)	3 (+)	0 (-)	1 (+)	2 (+)
Esterase (C 4)	4 (+)	3 (+)	3 (+)	4 (+)	4 (+)
Esterase Lipase (C 8)	4 (+)	3 (+)	3 (+)	3 (+)	2 (+)
Lipase (C 14)	1 (+)	0 (-)	1 (+)	2 (+)	0 (-)
Leucine arylamidase	3 (+)	1 (+)	3 (+)	5 (+)	1 (+)
Valine arylamidase	2 (+)	0 (-)	1 (+)	2 (+)	0 (-)
Cystine arylamidase	0 (-)	0 (-)	0 (-)	1 (+)	1 (+)
Trypsin	0 (-)	1 (+)	0 (-)	0 (-)	0 (-)
α-chymotrypsin	0 (-)	2 (+)	0 (-)	1 (+)	0 (-)
Acid phosphatase	5 (+)	0 (-)	0 (-)	2 (+)	3 (+)
Naphthol-AS-BI-phosphohydrolase	4 (+)	0 (-)	0 (-)	0 (-)	0 (-)
α -galactosidase	0 (-)	0 (-)	0 (-)	1 (+)	0 (-)
ß-galactosidase	1 (+)	0 (-)	1(+)	2 (+)	2 (+)
ß -glucuronidase	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
α -glucosidase	3 (+)	0 (-)	0 (-)	0 (-)	5 (+)
ß-glucosidase	2 (+)	0 (-)	0 (-)	1 (+)	0 (-)
N-acetyl-ß-glucosaminidase	0 (-)	0 (-)	0 (-)	1 (+)	0 (-)
α -mannosidase	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
α -fucosidase	0 (-)	0 (-)	0 (-)	1 (+)	0 (-)

Number indicates colour intensity which is proportional to concentration of respective enzyme presence. (+) = Enzymatic activity detected; (-) = Non-detectable.

molecular weight organic compounds are immediate precursors in the synthesis of proteins and participate in many pathways of microbial cell metabolism (Mudryk and Podgorska, 2006). Proteases such as the leucine arylaminase were also synthesized very intensively by the isolates. Jones and Lock (1989) reported that the level of leucine arylaminase activity is a good measure of the proteolytic activity of bacteria as it is a peptide bond hydrolysing enzyme.

Lipolytic enzymes are actively released or exported by living microorganism as free enzymes after the cell lysis which is capable of attacking emulsified mono-, di-and triglycerides and splitting them with the yield of glycerol and fatty acid residues (Mudryk and Podgorska, 2006). In this study, there were 20% of the isolates (SL4) showed phospholipase activity. The lipolytic activities of the isolates against short-chain fatty acids might be higher compared to the long-chain fatty acids as only 60% of the isolates were able to produce lipase which hydrolyses long-chain fatty acids in comparison that 100% of the isolates were able to produce esterase which hydrolyse short-chain fatty acids.

The soil isolates in this study showed a high potential of alkaline phosphatase activity demonstrated by the API ZYM assay. Mudryk and Skorczewski (2006) noted that phosphatases are actively synthesised by those isolates, which have a very efficient system of transporting phosphorus into their cells. He also reviewed that phosphatase-producing capacity was shown by many species of heterotrophic bacteria mainly from the genera *Pseudomonas*, *Chromobacterium*, *Bacillus*, and *Flavobacterium*. In present study, SL1 that might belong to the *Bacillus* species were found to have higher alkaline phosphatase and acid phosphatase activity.

This initial study will be followed up with identification of bacterial species using 16S rRNA gene analysis technique. This technique may provide information in terms of the community structure, diversity, evolution and taxonomy of microbial flora in the tin mining area. The sequencing information may also explain the discrepancy between phenotypic identification and the antibiotic susceptibility of the various isolates (Srivastava et al., 2008). These novel isolates will allow the exploration of new antibacterial agent and industrial enzyme with enhance prowess.

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REFERENCES

Ahmed N, Uzair B, Ayaz S, Ahmed VU (2008). Antibacterial activity of marine bacteria from Arabian Sea of Pakistan. Internet J. Microbiol., 4 (2): 1-11.

Cain BD, Norton PJ, Eubanks W, Nick HS, Allen CM (1993). Amplification of the bacA gene confers bacitracin resistance to *Escherichia coli*. J. Bacteriol., 175: 3784-3789.

Christopher W (2003). Antibiotics Actions, Origins, Resistance. U.S.A: ASM Press.

Dohren VH (1995). Peptides: In: Genetics and Biochemistry of antibiotic production (ed. Vining LC and Stuttard C). Newton MA. Butterworth-Heinemann, p. 129-171.

Ellaiah PK, Adinarayana SV, Pardhasaradhi, Srinivasula B (2002). Isolation of Alkaline protease producing bacteria from Visakhapatnam soil. Ind. J. Microbial., 42: 173-175.

Farah S, Samia A, Zobia Y, Sheikh AR (2009). Comparative Study of Two Bacteriocins Produced by Representative Indigenous Soil Bacteria. Pak. J. Pharm. Sc., 22 (3): 252-258.

Fariha H, Samiullah K, Aamer AS, Abdul H (2009). Production of Antibacterial Compounds by Free and Immobilized *Bacillus pumilus* SAF1. Pak. J. Bot., 41(3): 1499-1510.

Folasade M, Olajuyigbe, Joshua OA (2005). Production dynamics of extracellular protease from Bacillus species. Afr. J. Biotechnol., 4 (8): 776-779.

James GC, Natalie S (2008). Microbiology A Laboratory Manual (8th ed.).U.S: Pearson Education, Inc.

Jones SE, Lock, MA (1989). Hydrolytic extracellular enzyme activity in heterotrophic biofilms from contrasting rivers. Freshwat. Biol., 22: 289-296.

Jos M, Raaijmakers Maria V, Jorge T (2002). Antibiotic production by bacterial biocontrol agents. Antonie van Leeuwenhoek, 81: 537–547.

Liasi SA, Azmi TI, Hassan MD, Shuhaimi M, Rosfariza M, Ariff AB (2009). Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu. Malaysian J. Microbiol., 5(1): 33-37.

Magdi AM, Francis, FH, Moustafa AN. Mohamed SA (2009). Production of Protease in Low-Cost Medium by *Bacillus subtilis* KO Strain. Global J. Biotechnol. Biochem., 4(2): 132-137.

Maria LD, Vind J, Oxenball, Svendsen A, Patkar S (2007). Phospholipases and their industrial applications. Appl. Microbiol. Biotechnol., 74: 290–300.

Mendo S, Faustino NA, Sarmento AC, Amado F, Moir AJ (2004). Purification and characterization of a new peptide antibiotic produced by a thermotolerant *Bacillus licheniformis* strain. Biotechnol. Lett., 26: 115-119.

Mudryk ZJ (2002). Antibiotic Resistance among Bacteria Inhabiting Surface and Subsurface Water Layers in Estuarine Lake Gardno. Polish J. Environ. Stud., 11(4): 401-406.

Mudryk ZJ (2004). Decomposition of organic and solubilization of inorganic phosphorus compounds by bacteria isolated from a marine sandy beach. Mar. Biol., 145: 1227.

Mudryk ZJ, Podgorska B (2006). Enzymatic Activity of Bacterial Strains Isolated from Marine Beach Sediments. Polish J. Environ. Stud., 15(3): 441-448.

Mudryk ZJ, Skorczewski P (2006). Enzymatic activity and Degradation of Organic Macromolecules by Neustonic and Planktonic Bacteria in an Estuarine Lake. Polish J. Ecol., 54: 13–14.

Palvannan T, Boopathy R (2005). Phosphatidylinositol-Specific Phospholipase C Production from *Bacillus thuringiensis* Serovar. kurstaki using Potato-Based Media. World J. Microbiol. Biotechnol., 21 (6-7): 1153-1155.

Pollock TJ, Thorne L, Yamazaki M, Mikolajczak, MJ, Armentrout RW (1994). Mechanism of bacitracin resistance in gram-negative bacteria that synthesize exopolysaccharides. J. Bacteriol., 176 (20): 6229-6237.

Robert AP, Lorraine F, Walter M, Ronald MR (2009). Laboratory exercises in Microbiology (3rd ed.). U.S: John Wiley and Sons, Inc.

Schallmey M, Singh A, Ward OP (2004). Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol., 50: 1-17.

Srivastava S, Singh V, Kumar V, Verma PC, Srivastava R, Basu V (2008). Identification of regulatory elements in 16S rRNA gene of *Acinetobacter* species isolated from water sample. Bioinformation, 3(4): 173-176.

Talaro KP (2009). Foundation in Microbiology (7 ed.). New York:

Tambekar DH, Kalikar MV, Shinde RS, Vanjari LB, Pawar RG (2009). Isolation and Characterization of Multiple Enzyme Producer *Bacillus* Species from Saline Belt of Purna River. J. Appl. Sci. Res., 5(8): 1064-1066.

Vijendra KM, Ashok K (2009). Impact of Metal Nanoparticles on the Plant Growth Promoting Rhizobacteria. Dig. J. Nanomater. Biostructures, 4(3): 587 – 592.

Walczak M, Donderski W (2004). Antibiotic Sensitivity of Neustonic Bacteria in Lake Jeziorak Mały. Polish J. Envi. Stud., 13(4): 429-434.

Willey JM, Sherwood LM, Woolverton CJ (2008). Prescott, Harley and Klein's Microbiology (7th ed.). New York: McGraw-Hill.