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Isolation and Purification of Bacterial Strains from Treatment Plants for Effective and Efficient Bioconversion of Domestic Wastewater Sludge

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Abstract: Forty six bacterial strains were isolated from nine different sources in four treatment plants namely Indah Water Konsortium (IWK) sewage treatment plant, International Islamic University Malaysia (IIUM) treatment plant-1,-2 and –3 to evaluate the bioconversion process in terms of efficient biodegradation and bioseparation. The bacterial strains isolated were found to be 52.2% (24 isolates) and 47.8% (22 isolates) in the IWK and IIUM treatment plants respectively. The results showed that the higher microbial population (9-10x10^4 cfu mL^{-1}) was observed in the secondary clarifier of IWK treatment plant. Only the gram-staining identification was done in the strains isolated from IWK treatment plant not to be determined from IIUM. Among the isolates from IWK, 10 isolates of gram-positive bacillus (GPB) and gram-positive cocci (GPC), 10 isolates of gram-negative bacillus (GNB) and rest were both or undetermined. Gram-negative cocci (GNC) were not found in the isolates from IWK.

Key words: Bacteria, domestic wastewater sludge, isolation, bioconversion

INTRODUCTION

Sewage treatment plants are artificial ecosystems in which the biodegradation of organic pollutants is carried out mainly by bacterial communities and producing excess sludge. This excess sludge presents serious disposal problems due to its huge quantity and toxicity. In Malaysia, about 3.8 million cubic meters of domestic wastewater sludge is produced annually by Indah Water Konsortium (IWK) and its management cost is estimated to RM 1 billion[1]. Effective and efficient developed bioconversion process through potential microbes in terms of biodegradation and bioseparations as well as the environmentally friendly are the main concern of IWK.

To achieve the goal, the effective bioconversion process is being considered to treat the wastewater sludge through potential microorganisms involved. In this process, the potential bacterial strains isolated from different wastewater treatment plants are involved to degrade the dissolved and suspended organic substances in sludge and enhance the biodegradation, biosolids reduction and bioseparation processes. Among microorganisms participating in activated sludge communities bacterial isolates have been attracting increasing attention in terms of faster growth, high resistance to contaminate and adaptation compared to other organisms from biotechnological point of view. In fact bacteria of this consortia are known to be involved in biodegradation of a number of different pollutants and processes such as waste materials[2], denitrification[3], wastewater treatment[4], biphenyl (BP) and chlorinated BPs[5], phenol[6] crude oil and lubricant[7,8]. Therefore the present study was undertaken to isolate, purify and identify (gram-staining) the bacterial strains for effective bioconversion of wastewater sludge through biodegradation and bioseparation under natural conditions.

MATERIALS AND METHODS

Sample collection: Nine samples especially wastewater and wastewater sludge were collected from different sources in four treatment plants. The treatment plants are: Indah Water Konsotium (IWK) sewage treatment plant, Kuala Lumpur, IIUM treatment plant-1, -2 and -3, Gombak Campus, Kuala Lumpur. Samples were stored at 4^0C and analyzed within 24 h.

Isolation and purification of bacteria: The brain heart agar (BHA) medium was used to isolate the bacterial strains from the samples. The cell counting technique in agar plates was followed to determine the population density for each sample. A series of dilutions were made to reduce the cells in the samples. One ml of

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diluted sample was spreaded onto the surface of BHA medium in the petri dishes and incubated at 37^0C and allowed to grow for 24 h. Single developed colony was picked on the BHA plates and subcultured to purification. Pure bacterial strains were obtained after successive transfer of individual colony in BHA plates and incubated for 24 h at 37^0C temperature. A partial identification only the gram staining was observed in the selected isolates.

RESULTS

A total of 46 bacterial strains were isolated from nine different sources in four treatment plants (Table 1). The bacterial community in the brain heart agar (BHA) plates from different sources in treatment plants for isolation is shown in Fig. 1. The single bacterial colony was cultured from microbial community in BHA plate. Among them 24 strains were isolated from IWK treatment plant and 22 strains from IIUM treatment plants (three plants). The distribution of isolated bacterial strains was 52.2% and 47.8% (TP-1: 13%, TP-2: 19.6%, TP-3: 15.2%) in the IWK and IIUM treatment plants, respectively. The maximum population density (9-10x10^4 cfu mL^{-1}) of bacterial communities was counted in the influent of secondary clarifier of IWK treatment plant followed by the IIUM treatment plant-3 (6-7x10^4 cfu mL^{-1}), -2 (2.5-3x10^4 cfu mL^{-1}) and -1 (2.2-2.5x10^4 cfu mL^{-1}), respectively. The lowest density (4-5x10^3 cfu mL^{-1}) was found in the effluent sample in IWK plant. The representative strains of bacterial pure culture isolated from different sources in four treatment plants after 24 hrs of incubation at 37^0C are shown in Fig. 2.

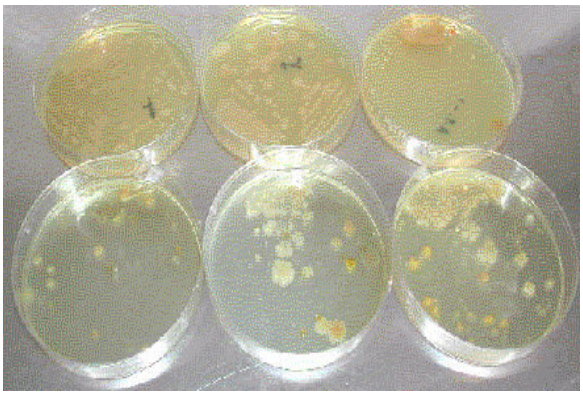

Fig. 1: Microbial community from different treatment plants in brain heart agar (BHA) plates for isolation of pure bacterial cultures

Most of the colony pigments in BHA media were appeared in light creamy and some isolates were white and yellow in the same media.

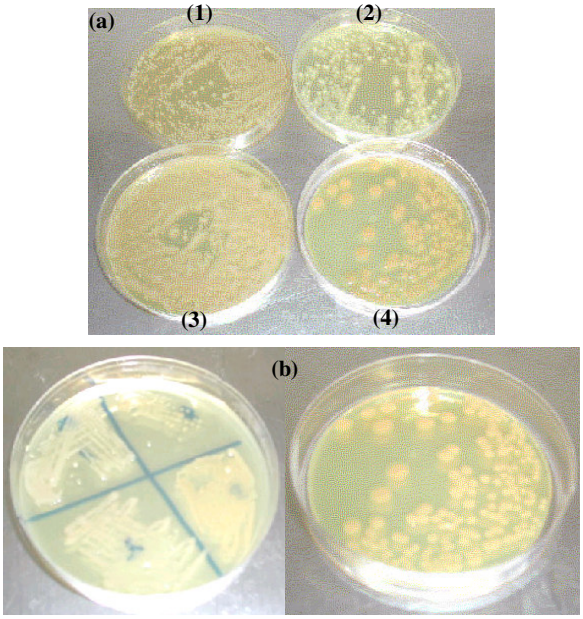

Fig. 2: Bacterial pure culture as the representative strains isolated from different sources in 4 treatment plants. (a-1): IWK1010; (a-2): IWK2014; (a-3): IIUM-I101; (a-4): IIUM-E322; (b) Single culture in BHA plate

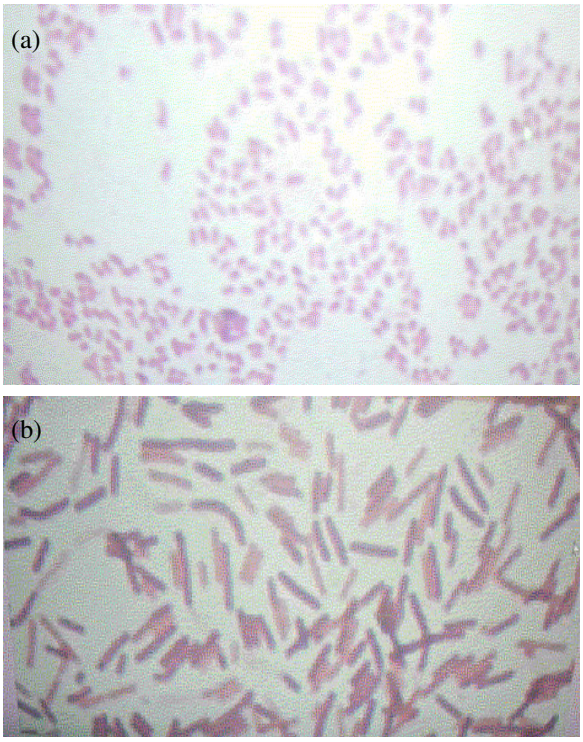

Fig. 3: Microscopic view of bacteria identified as the (a) Gram-positive cocci (round shape); (b) Gram-positive bacillus (rod shape)

Table 1: Bacterial strains isolated from different treatment plants in Malaysia for effective bioconversion of domestic wastewater sludge

Sources			Isolation			Identification	Total isolates
			Code number	Population, cfu/ml	No. of isolates	Gram-staining*	
IWK treatment plant	Secondary clarifier		IWK1001 IWK1002 IWK1003 IWK1004 IWK1005 IWK1006 IWK1007 IWK1008 IWK1009 IWK10010	9-10x10^4	10	GPB GPB GPC GNB GNB GNB GNB GNB GPC UN	24
	Aeration tank		IWK2001 IWK2002 IWK2003 IWK2004 IWK2005 IWK2006 IWK2007 IWK2008 IWK2009 IWK20010	7-8x10^4	10	GPC GPC GPB+GNB GNB GPC GPC GNB GNB GNB GNB	
	Effluent		IWK3001 IWK3002 IWK3003 IWK3004	4-5x10^3	4	GPC UD UD GPC	
	Total				24		
IIUM Treatment Plants	Treatment plant-1	Influent	IIUM-I101 IIUM-I102	10-12x10^3	2	GPB GPC	22
		Effluent	IIUM-E103 IIUM-E104 IIUM-E105 IIUM-E106	2.2-2.5x10^4	4	GNC GPB GPB UD	
	Total				6		
	Treatment plant-2	Influent	IIUM-I201 IIUM-I202 IIUM-I203 IIUM-I204	10-12x10^3	4	GPB GPB GNC UD	
		Effluent	IIUM-E209 IIUM-E205 IIUM-E206 IIUM-E207 IIUM-E208	2.5-3x10^4	5	GPB GPC GNB GNB GNB	
	Total				9		
	Treatment plant-3	Influent	IIUM-I301 IIUM-I302 IIUM-I303 IIUM-I304	10-12x10^3	4	GPC GPC GPB GNC	
		Effluent	IIUM-E305 IIUM-E306 IIUM-E307	6-7x10^4	3	GPB GNC GNB GNB	
	Total				7		
Total							46

The gram-staining identification was observed to the strains isolated from all treatment plants. The gram-staining test such as gram-positive bacillus (GPB), gram-positive cocci (GPC), gram-negative bacillus (GNB) and gram-negative cocci (GNC) was determined in the bacterial strains that isolated both treatment plants. Figure 3 showed the gram-staining identification among the isolates from treatment plant. The total isolates (46 strains) were found to be 22 strains of GPB and GPC, 19 strains of GNP and GNC, 1 of both and 4 strains were undetermined. No result was found as the GNC in IWK treatment plant. The results indicated that the bioconversion of wastewater sludge might be effective through gram-positive and/or gram-negative strain especially isolated from IWK treatment plant as well as the IIUM treatment plants.

DISCUSSION

The microbial population have been measured in two treatment plants which were the average concentration of $1.7x10^3$ cfu m^{-3} of mesophilic and $2.1x10^2$ cfu m^{-3} of TSA-SB bacteria (bacteria associated with certain water born virulence factors) in the aeration tank of the activated sludge treatment plants and in the fixed film reactor $3x10^3$ cfu m^{-3} of mesophilic and 730 cfu m^{-3} of TSA-SB bacteria were found[9]. The present study showed the higher density of bacterial community in the treatment plants compared to the literature.

Different applications of bacterial community isolated from wastewater sludge are employed globally. Seven groups of 26 morphological types of filamentous bacteria were isolated, cultivated and identified from activated sludge to achieve a better understanding of the complex phenomena of sludge bulking and foaming that influences the settleability and dewaterability of sludge[10]. A total of 165 denitrifying bacteria were isolated from activated sludge for polyphosphate accumulation and denitrification in biological removal of nutrient[11]. Several authors have been studied on biodiversity of bacterial community isolated from activated sludge[12-14]. So far no study was done on bioconversion of wastewater sludge with the potential bacterial culture in terms of effective biodegradability, dewaterability and settleability. Recently, filamentous fungi were isolated mainly from wastewater treatment plants for sludge bioconversion in liquid state (treatment)[15,16] and solid-state (compost) respectively[17].

The results presented in this study indicated that 46 strains were isolated and purified from different sources in four treatment plants. The identification of gram-staining level was conducted in the isolates from IWK treatment plant. The higher microbial population was observed in the secondary clarifier in IWK treatment plant compared to other treatment plants. The potential bacterial strains through screening might enhance biodegradability and dewaterability of wastewater sludge considering its faster growth, resistance to contaminate and adaptation that could contribute the new development of biological treatment processes in future solutions.

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