Journal of Global Biosciences
ISSN 2320-1355
Volume 6, Number 9, 2017, pp. 5212-5221
Website: www.mutagens.co.in

Research Paper

OCCURRENCE AND BIODEGRADATION OF KEROSENE BY AQUATIC PHYCOMYCETES FROM SOME WATER BODIES IN A SEMI-ARID AREA OF NIGERIA

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Abstract

This study was carried out on the occurrence of aquatic phycomycetes and biodegradation of kerosene by some aquatic phycomycetes isolates that occurred in some polluted water bodies in Maiduguri. Isolation was done by baiting method using hemp seed as bait while identification was done by following standard protocols. Abilities of three species of aquatic phycomycetes to degrade kerosene were tested on PDA medium supplemented with 1%, 5% and 10% (V/V) kerosene. A variety of aquatic phycomycetes species were isolated from the three freshwater bodies studied. The Lagos Bridge and Coca-cola sections of the River Ngadda were found to contain more isolates than the Lake Alau location of the sampling sites. Considering the anthropogenic environmental activities in the three locations, the Lagos Bridge and Coca-cola areas are more polluted than the Alau Dam location which is a source of water for treatment into portable drinking water by the Borno State Government. The more occurrences of aquatic phycomycetes in the more polluted areas could implicate these isolates as pollution indicators. Three aquatic phycomycetes species namely *Saprolegnia bhargavi*, *Blastocladiella variabilis* and *Allomyces recurvus* showed good abilities in degrading kerosene supplemented PDA with the highest growth presented by *Saprolegnia bhargavi* in 5% kerosene supplemented PDA culture medium. The abilities of the three test aquatic phycomycetes species to grow in a medium supplemented with kerosene shows they can biodegrade kerosene and could be further considered as a bioremediation agent.

Key words: ***Aquatic phycomycetes, biodegradation, baiting method, kerosene and polluted***.

INTRODUCTION

Aquatic phycomycetes are a group of aquatic fungi that mainly belong to members of Chytridiomycetes and Oomycetes, though mainly aquatic, some species are found on moist soil. Chytridiomycetes and Oomycetes are separated on the basis of flagella being uniflagellate and biflagellate respectively (Khulbe, 2001). Aquatic phycomycetes form a group of fungi that is readily isolated using various plant materials as "bait" Marano *et al.,* (2008), Nascimentol *et al.,* (2011) and Trifa and Adiba (2011). Many ecological studies of aquatic phycomycetes have been studied worldwide (Alabi, 1971; Willoughby, 1974; Steiow, 1998; Marano *et a., (* 2008). Reports of the occurrence of aquatic phycomycetes in Nigeria generally is scanty and have not surveyed their application in general due to the very few researchers interested in these group of primitive fungi. These fungi contribute to the energy flow and productivity of aquatic and semi aquatic ecosystem by their active role in the utilization and bio deterioration of a variety of organic and inorganic materials (Khulbe, 2001). Environmental pollution due to oil spill is one of the most common problems along the coastal region but in the hinterlands like the water ways of the savannah and semi arid zones, oil pollution is also introduced by human activities of washing automobiles and engines, domestic utensils and occasional discharges by fuel tanker drivers due to accidents . Petroleum contamination effects on microbial population are various (Ahearn and Meyers, 1976). Certain microbes show increase in population due to use of petroleum hydrocarbons as nutrients (Westlake *et al.,* 1974). Such species are commonly being used for remediation of contaminated site. Although oil spills contaminates living and non livings, microorganisms especially fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have mechanisms for the elimination of spilled oil from the environment (Dibble and Bartha 1979; Atlas 1995). Reviews on Fungi in bioremediation of crude oil across the world (Nilanjana and Preethy, 2011; Jahangeer and Vikram 2013) and in Nigeria (Obire and Putheti, 2009) recorded yeasts and filamentous fungi as fungi biodegraders of crude oil. None of the reviewers encountered in this study reported aquatic phycomycetes as biodegraders of crude oil or its fraction. The present work therefore aims at isolation and identification of aquatic phycomycetes from some polluted freshwater bodies and their role in the degradation of kerosene as a pollutant in the semi arid area of Maiduguri, Nigeria.

MATERIALS AND METHODS

Study Location and Sampling Sites

The study was conducted in Maiduguri which is located in the semi-arid area of North-eastern Nigeria. The area is characterized by short rainy season (3 – 4 months) and long dry season (8 – 9 months). Mean ambient temperature is 31 ^{o}C by August but gets as high as 40 ^{o}C or more by April to May (Alaku and Moruppa, 1988). The water samples were collected from three sampling sites in the Maiduguri area namely Alo dam, River Ngadda by Coca-cola company area and River Ngadda by Lagos bridge.

Water Sample Collection, Transportation And Storage

Water samples from experimental sampling sites (Alau dam, River Ngadda by Coca-cola area and River Ngadda by Lagos bridge) were collected in sterile 100mls and 200mls bottles at the littoral zones (shorelines) of these water bodies in the month of April 2017. The sampling bottles were opened inside the water and immediately capped before bringing them out of the water to avoid contamination. All collected water samples were labelled with different code for analysis purpose. Water samples were then transported to the laboratory in Biological Sciences department of University of Maiduguri, in a plastic cooler with ice packs maintained at a temperature of 4^{o}C and were analysed as soon as practicable on the day of collection between 2-6 hours.

Isolation and identification of aquatic phycomycetes

Aquatic phycomycetes species were isolated from the water samples collected from the three sampling sites above using hemp seeds (*Cannabis sativa*) as bait in 20cm Petri-dishes as used by Marano *et. al*., (2008) and Nascimentol *et. al*., (2011). Observations of growth of the baited aquatic phycomycetes were made for four to seven days. and in some cases for up to 3 weeks. Each Petri dish was considered a sample unit according to the method of Marano *et al*., (2008) Nascimental *et al*., (2011) and Trifa and Adiba (2011). For the determination fungal population, the aquatic fungal species appearing on one plate was counted as one colony according to the method of Trifa and Adiba (2011). Colonies appearing on baited seeds were separated based on closely observed morphological differences like colour, length of the hyphae, concentration of hyphae around the seed and in conclusion differentiation into species was based on morphological differences of zoosporangia and hyphae. Baited seeds with aquatic phycomycetes isolated showing similar morphological characteristic were pulled together into Petri dishes containing sterile distilled water to which anti-biotic had been added to suppress bacteria growth and observed directly under the compound light microscope for identification (Trifa and Adiba, (2011),. Marano *et. al*., (2008) and Nascimentol *et. al*., (2011), alternatively, observation of a pin head of hyphae stained with lactophenol cotton blue was also made under a compound light microscope to support identification. The identification of isolated strains was done as per guidelines of Khulbi, (2001) and Ali, (2007). Appropriately identified isolates were recorded as present for respective sampling sites and used to determine the frequency of occurrence of identified isolates. Three of the appropriately identified isolates that had been pulled together based on observed morphological similarities were further maintained in sterile distilled water with drops of 0.3% streptomycin sulphate to suppress bacteria growth and subsequently used for the biodegradation experiment. The test aquatic phycomycetes species isolated and used for the biodegradation of kerosine supplemented potatoes dextrose agar were *Allomyces recurvus* from Alo dam, *Blastocladiella variabilis* from River Ngadda by Lagos bridge and *Saprolegnia bhargavi* from River Ngadda by Coca-cola area all within Maiduguri metropolitan council.

Determination of the Abilities of Test Aquatic Phycomycetes Isolates to Degrade Kerosene Supplemented PDA Culture Medium

Studies were carried out to determine the abilities of test species of aquatic phycomycetes isolates to utilize kerosine contaminated Potatoes Dextrose Agar according to the method used by Sakineh*. et al*., (2012) . Three concentrations of kerosine supplemented PDA media (1%, 5%, and 10%(v/v) of kerosine) and one control PDA media were each prepared in 500ml conical flasks according to manufacturers instruction and sterilized. The carbon source which is kerosine was sterilised by filteration with whatman filter paper while the PDA was by standard autoclave method. The three test species of aquatic phycomycetes isolated from the aquatic sampling sites were then grown separately in the PDA (Potatoes Dextrose Agar) as control and three differrent percentages of Kerosine supplemented PDA with drops of streptomycin sulphate (0.3g in 1000mls) to suppress bacteria growth in triplicates and allowed to set or solidify up to 45^{0}C and subsequently incubated at room temperature and observed daily between 1-7 days for growth by measuring the daily colony diameter in centimetres. The aquatic phycomycetes isolated and used for the biodegradation of kerosine supplemented PDA were *Allomyces recurvus* from Alo dam, *Blastocladiella variabilis* from River Ngadda by Lagos bridge and *Saprolegnia bhargavi* from River Ngadda by Coca-cola area.

RESULTS

Quantitative Count of Aquatic Phycomycetes Baited on Hemp Seed

Colonistion of aquatic phycomycetes on hemp seed as bait was slow in all the water samples from the different sampling points in the first week of the experiment but peaked after three weeks of incubation. Counts of aquatic phycomycetes emerging on baited hemp seeds after three weeks as shown in Table 1 revealed that the sampling points on River Ngadda by Lagos bridge had the highest count totalling 37 colonies followed by that of River Ngadda by Coca-cola area with 36 colonies and the least count was presented by Alau dam with 23 colonies

Table 1:Quantitative Counts of Aquatic Phycomycetes in Sampling Sites in the Study Area

	Counts of aquatic phycomycetes in different sampling sites								
Sampling site	Lagos bridge			Coca cola			Alau dam		
Sampling points	A	B	C	A	B	C	A	B	C
Replicate 1 (3 p5hspsp)	3	5	4	2	4	3	2		2
Replicate 2 (3 p5hspsp)	2	6	5	4	6	2	3	4	5
Replicate 3 (3 p5hspsp)	4	2	6	5	5	5		3	4
Total	9	13	15	11	15	10	5	7	11
Total/site			37			36			23

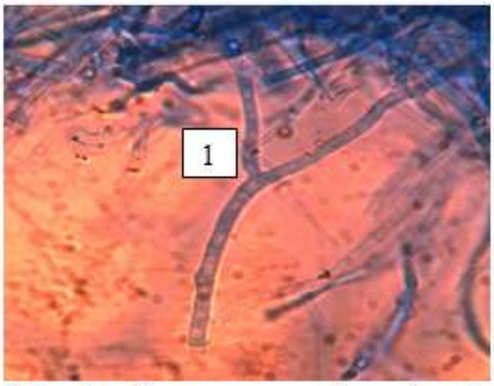
Note; 3p5hspsp = Three Petri-dishes containing five hempseeds each per sampling point in each sampling site.

Qualitative Occurrence Of Aquatic Phycomycetes In Different Sampling Sites

Ten species of aquatic phycomycetes were isolated from the three sampling sites as shown in Table 2 and they included *Allomyces anomalus, Allomyces arburscular, Allomyces recurvus, Brevilegnia diclina, Saprolegnia bhargavi, Saprolengnia ferax, Protoachyla oryzae, Protoachyla paradoxa, Blastocladiela variabilis* and *Aphanomyces helicoides.* Table 2 also shows frequency of occurrence of these isolates in the three sampling sites with *Allomyces anomalus* occurring in all three sampling sites (100%) while *Allomyces arbuscular*, *Allomyces recurvus*, *Blastocladiella variabilis* and *Saprolegnia bhargavi* occurred in two of the three sampling sites (66.66%). Table 2 also shows that River Ngadda by Lagos bridge and Coca-cola area recorded five(5) and four(4) isolate occurrences respectively while Alau dam recorded the highest isolate occurrence of seven(7). Micrograph of three of the isolates namely *Allomyces anomalus* (Plates 1 &2), *Allomyces arbuscular* (Plate 3) and *Brevilegnia diclina* (Plate 4) are also presented with distinctive features.

TABLE 2: Qualitative Occurrence of Aquatic Phycomycetes in the Sampling Sites

Aquatic phycomycetes isolates	Presence or absence in sampling sites				
	Lagos bridge	Coca-cola	Alau dam	Total	Frequency (%)
Allomyces anomalus	+	+	+	3	100
Allomyces arbuscular	-	+	+	2	66.66
Allomyces recurvus	+	-	+	2	66.66
Aphanomyces helicoides	+	-	-	1	33.33
Blastocladiella variabilis	+	-	+	2	66.66
Brevilegnia diclina	-	-	+	1	33.33
Protoachyla oryzae	-	-	+	1	33.33
Protoachyla paradoxa	-	-	+	1	33.33
Saprolegnia bhargavi	+	+	-	2	66.66
Saprolegnia ferax	-	+	-	1	33.33
Total	5	4	7		



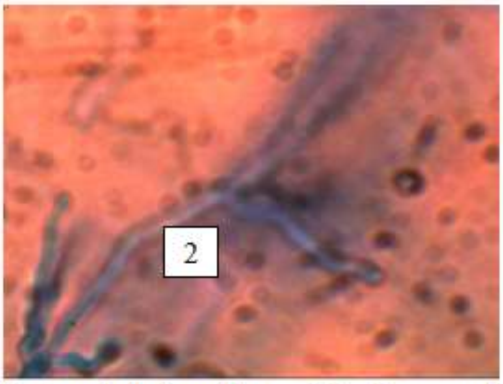
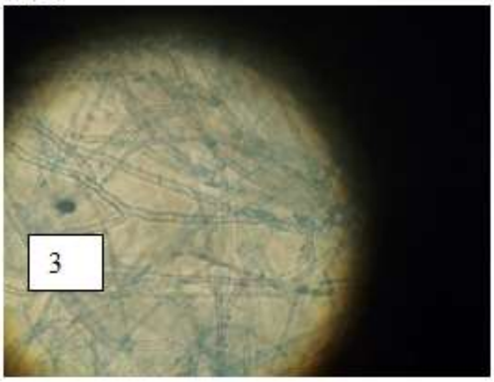


Plate 1: *Allomyces anomalus* showing fertile terminal branching of hyphae and irregular surface captured on microscope slide stained with lactophenol cotton blue
Plate 2: *Allomyces anomalus* showing fertile terminal branching of hyphae, irregular surface and resting abundant sporangia captured on microscope slide stained with lactophenol cotton blue.



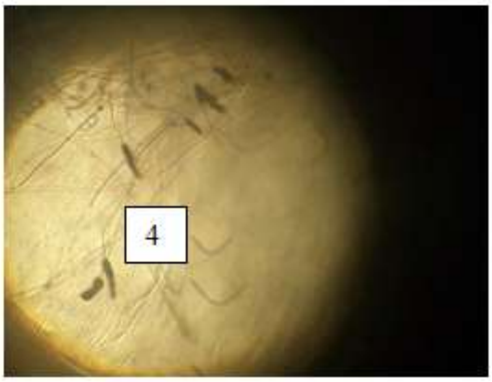


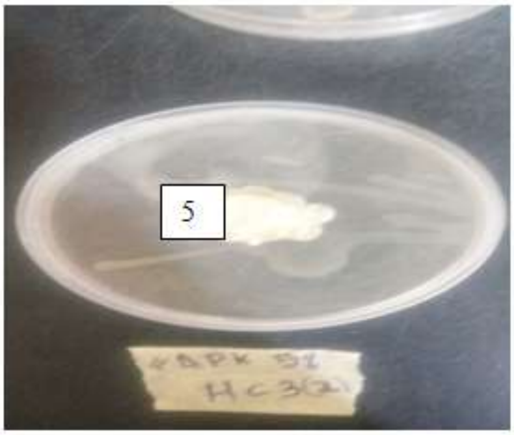
Plate 3: Micrograph of *Allomyces arbuscular* showing branching pattern of hyphae, thick walled resting sporangium and pseudoseptate hyphae
Plate 4: Micrograph of *Brevilegnia diclina* showing terminal matured zoosporangium with liberation of zoospores

Biodegradation of Kerosene by Some Aquatic Phycomycetes Isolates from Three Freshwater Bodies in Maiduguri

The abilities of three aquatic phycomycetes to utilize kerosene supplemented PDA culture media was tested. The three isolates tested included *Saprolegnia bhargavi, Blastocladiella variabilis and Allomyces recurvus*. The results of colony diameter of the three test isolates on a daily basis for three days are shown in tables 3. The tables shows best progressive growth of the test isolates on the control culture medium containing PDA without supplement of kerosene. The growth diameter for all the isolates on the third day on the control PDA culture medium without kerosene supplement were approximately 2.45cm, 2.7cm and 2.0cm for isolates *Saprolegnia bhargavi, Blastocladiella variabilis* and *Allomyces recurvus* respectively translating to a mean diameter growth of 2.38cm as shown in Table 3. *Saprolengnia bhargavi* showed the best growth on PDA supplemented with kerosene on 5% kerosene supplemented PDA after three days with a growth diameter of 2.30cm as shown in plate 5, while the same isolate, *Saprolegnia bhargavi*, grown on 10% kerosene supplemented PDA showed a smaller minimal growth diameter of 0.4cm as shown in plate 6. *Allomyces recurvus* as shown in plate 7 recorded the least minimal colony diameter of 0.2cm on both 5% and 10% kerosene supplemented PDA culture medium. Blastocladiella as also shown in Plate 8 recorded a growth diameter of 0.4cm on the PDA supplemented with 5% kerosene on the third day.

Table 3: Daily average growth diameter of aquatic phycomycetes in oil contaminated PDA medium

No of days	Test isolate	Concentration of kerosene(v/v) and growth colony diameter (cm)			
		Control	1%	5%	10%
Day 1	*Allomyces recurvus*	0.55	0.00	000	0.00
	Blastocladiella variabilis	0.90	0.00	0.00	0.00
	Saprolegnia bhargavi	0.90	0.00	0.00	0.00
Day 2	*Allomyces recurvus*	1.40	0.00	0.10	0.10
	Blastocladiella variabilis	1.65	0.00	0.10	0.10
	Saprolegnia bhargavi	1.55	0.00	2.00	0.10
Day 3	*Allomyces recurvus*	2.00	0.00	0.20	0.20
	Blastocladiella variabilis	2.70	0.00	0.40	0.30
	Saprolegnia bhargavi	2.45	0.00	2.30	0.40
Day3 mean		2.38	0.00	0.96	0.30



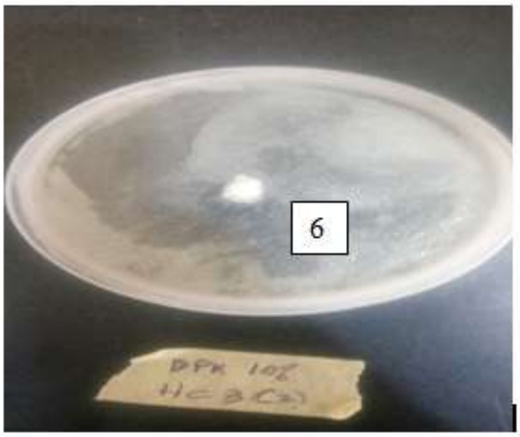
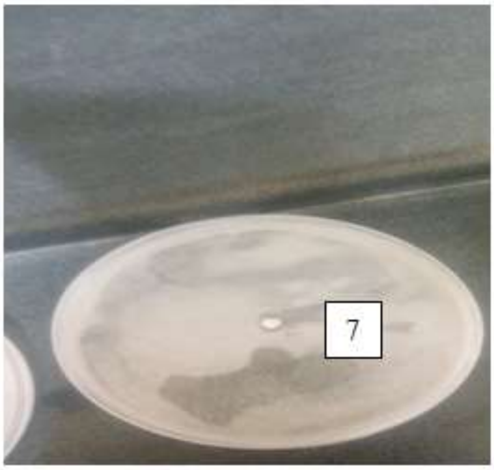


Plate 5 : Cultural appearance of *Saprolegnia bhargavi* on day 3 on 5% kerosene supplemented PDA (2.3cm)
Plate 6: Cultural appearance of *Saprolegnia bhargavi* on day 3 on 10% kerosene supplemented PDA (0.4cm)



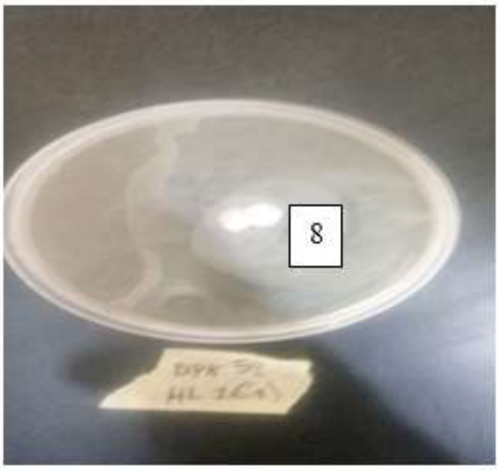


Plate 7 : Cultural appearance of *Allomyces recurvus* on day 3on 5% kerosene supplemented PDA (0.2cm)
Plate 8 : Cultural appearance of isolate *Blastocladiella variabilis* on day 3 on 5% kerosene supplemented PDA (0.4cm)

DISCUSSION

In the study of occurrence of aquatic phycomycetes in three freshwater bodies in Maiduguri, Ten (10) species of aquatic phycomycetes were isolated and identified, these isolates which included *Allomyces recurvus, Allomyces anomalus, Allomyces arbuscular, Saprolegnia bhargavi, Saprolegnia ferax, Protoachyla oryzae, blastocladulla variabilis* amongst others have been reportedly isolated in other water bodies by Nelson- Smith ,(1973). Water sample from River Ngadda by Lagos bridge and Coca-cola area had the highest number of isolate occurrences. This may imply that River Nggada by Lagos bridge and Coca-cola area are more polluted than the Alau Dam sampling site. This can safely be assumed because of the high level of human activity that introduces a lot of domestic and industrial wastes and accidental discharges into the water body and the activities of cattle herdsmen that bring their cattle to drink from the water body as opposed to the Alau Dam used as a source of water by the State water treatment plant for supply of portable water to the metropolis. The relative higher quantitative occurrence of isolates of aquatic phycomycetes in seemingly more polluted water bodies may implicate these isolates as indicators of water pollution. In furtherance of that, qualitattively, more species make up the lower number of isolates from the less polluted water body of Alau Dam while less species accounts for the higher number of isolates from the more polluted water bodies of River Ngadda by Lagos Bridge and Coca-cola area. This may imply that the polluted status of the water bodies restricted some species from growing while the ones that were able to adapt to the pollution status increased in popolation. This reasoning seems to also have been suggested by Ayodhya and Venkat (2013) in their study of diversity of aquatic fungi from Mula River at Pune city in India when they recorded higher fungal Taxa in stations that were unpolluted and with less anthropogenic activities while lower fungal Taxa were recorded in stations regarded as polluted with increased anthropogenic activities. Tsui *et al.* (2001) made a similar observation when they reported that aquatic fungi diversity gradually declines with increased water contamination.

A remarkable observation in the biodegradation experiment was that one of the isolates *Saprolegnia bhargavi* showed the best growth at 5% kerosene supplementation and showed lesser growth on 10% supplementation. Similar observation was reported by Adekunle and Adebambo (2007) in which the isolated *Rhizopus* species from the seed of *Detarium senegalense* showed the highest ability to degrade kerosene amongst *Aspergillus flavus, Aspergillus niger, Mucor* and *Talaromyces*. In support of the findings of this research, a study by Wemedo *et. al*., (2002) also recorded that the genera of fungi such as *Penicillium, Aspergillus and Rhizopus* are associated with kerosene- polluted soil. Oboh *et. al.,* (2006) also reported that all these isolates were able to grow on crude Petroleum as sole carbon and energy source when screened for hydrocarbon utilization. Similar results were also obtained by Sekineh *et. al.,* (2012) who reported that the growth rate of fungus shows that *Rhizopus* sp had the highest growth diameter in 5% kerosene contaminated PDA media culture and *Aspergillus niger* had the highest growth diameter in 20% kerosene while *Penicillin* sp had the lowest growth rate at all concentrations. It is worthy of note that most of the experiments of biodegradation of crude oil and its fractions reported in available literature involved filamentous fungi like *Aspergillus* sp, *Penicillium* sp and *Rhizopus* sp amongst others. Little or no report is available on the ability of aquatic phycomycetes to utilise crude oil and it's fractions as sole carbon and energy source particularly in Maiduguri located in the Arid zone of North Eastern part of Nigeria. Therefore the current research may be one of the pioneering efforts in that direction and in the arid zone region of North Eastern Nigeria..

CONCLUSION

The fact that test isolates of aquatic phycomycetes grew at varying degrees even though minimaly on kerosene supplemented PDA medium shows that they have the ability to degrade kerosene as pollutants. Thus they can be surveyed for use in the bioremediation of kerosene pollutant in polluted aquatic environment.

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