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CHARACTERIZATION OF MICRO FLORA FROM COAL MINES OF BALUCHISTAN, PAKISTAN

Tabeel tariq[1*], M.Adnan ateeq[1], Masood T.kiyani[1], Nazeer Ahmed [1], Farooq shahzad [1] M.Yousaf[1], I. A Sani[2], M. Murad[2],Asma Yousafzai[2], Nisar Ahmed[2],Dawood Shahid[2], Hummaira Sadaf[3].

[1]Institute of Microbiology CASVAB, University of Balochistan Quetta, Pakistan
[2]Department of Biotechnology, Balochistan University of Information Technology. Engineering and Management Sciences,Quetta,Pakistan
[3]Sardar Bahadur Khan,Women University, Quetta,Pakistan

Abstract:
The coal sites of our country Pakistan especially Baluchistan and their micro flora have not been defined properly. However, it is thought to harbour a number of microbes including harmful, non-harmful gram positive, gram negative, acidophilic, alkaliphilic, halophiles, and thermophiles within itself. The proposed study aims to seek a cultural and diversified cataloguing of the entire micro flora present in the coal sites of Mach, Baluchistan. The study spreads to a timeline of 3 months and samples will be collected from Mach coal mine. Moreover, workers, who are suffering from lung diseases in particular were, will also be subjected to analysis to assess probable correlation with microbes. Total of 150 samples were collected in which 50 of coal dust samples were collected with sterilized swabs. 50 samples of airborne debris from mines sewerage water where collected in pre-sterilized Duran bottles. Moreover 50 sputum mine worker samples were collected in sterilized stomacher bag for isolation and correlation studies. Out of 150 samples we isolate different genera of kingdom monera which are related to coal mines and environment. Isolated microbes includes harmful, non-harmful gram positive, gram negative,acidophile, alkaliphilic, halophiles sulphur oxidising and thermophiles bacteria's which perform different activities according to the atmosphere and offered nutrient's .obligate acidophilic, heterotropic ,aerobic motile ,gram negative bacteria are generally found. Acidiothiobacillus species is major among them. Beside it, Pseudomonas, flavobacteriu species, shigella specie, vibrio parahaemolyticuc, Staphylococcal aureus and Escherichia coli colonies are also obtained. Microscopy performs for colony morphology. During microscopy we found different shapes of bacteria's like rod, cocci, spring shape etc. Further more we perfume APi 20 kit test for some bacterial species and compile the results. The study concluded that coal mines are riche in microbial flora which is effective for coal miners and also for the GDP growth of the country. Somehow there are also harmful bacteria's but they do not produce great impact on miner.

Keywords: *coal mine microbial flora , microscopy, biochemical tests.*

Corresponding Author:
Tabeel tariq,
Institute of Microbiology CASVAB,
University of Balochistan,
Quetta, Pakistan.
E-Mail: tabeel.aryan@gmail.com

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INTRODUCTION:

Microbial world contributes to most of our ecosystem with them ranging from literally every locality of planet. Coal mines are also a prime site for various strains of microbes where there is a high probability of them coming in contact with humans working within. This in turn may lead to various diseases mainly related to lungs. Assessment of this microflora originating from coal mines in Baluchistan is the prime purpose of the study(1).

The realm's claim for coal, the ''black gold,'' has been on the improved earlier the jerk of the trade uprising. The coal withdrawal action is categorized by the group of huge volume of by-yields. One of the special effects is the rise in heavy metal solubility, which effects in the gathering of these toxic components in the atmosphere. The toxic sound effects of heavy metals primarily product from the contact of metals with proteins (enzymes) and reserve of high metabolic developments. (Mclaughlin et al., 1999; Giller et al., 1998). (2) Bacteria that are strong to heavy metals similarly show an significant part in biogeochemical pedalling of those metal ions (Issazadeh et al., 2013; Kumar et al., 2011).(3) Certain microbes have however modified to stand the existence of metals and practice them to raise, these contacts between microbes and metals have key ecological effects mainly in bioremediation. Over the past period the usage of genes programming 16SrDNA as molecular pointers has come to be a up-to-date method for microbial biologists present different views on the old-fashioned phenotypic cataloguing structure (Woese, 1987).
(4)There have been several revisions on heavy metal conflict of bacteria secluded from several territories (Shi et al., 2002).(5) Present have been various revisions on heavy metal struggle of bacteria secluded from different environments (Ezaka et al., 2011). Under state of affairs of metal strain, metal and antibiotic struggle in microorganism's maybe relief them to adjust faster by the spread of resistant factors than by mutation and natural selection (Silver and Misra, 1988). (6)Bacterial plasmids have genes that confer highly specific resistances to toxic heavy metals. For all toxic cation and anion, usually a diverse resistance structure be present, and these structures might be related organised on various resistance plasmids (Silver et al., 1989).
(7)The hereditary factors of struggle are often situated on plasmids (Cervantes et al., 1991). The aims of this revision is to separate, classify and illustrate heavy metals resistant bacteria from soil section in Bokaro coal mines which potency be useful in heavy metal bioremediation from the adulterated site. (Murthy,etal.,2014).

MATERIALS AND METHODS:

Survey of study;

Mach is a major metropolis in addition to union council of Bolan District in the Balochistan province of Pakistan. The town has an height above sea level of 1006 metres (3303 feet) and is located at 29°52'0N 67°19'60E, - some about 50 km (70 km by road) southeast of Quetta, the provincial capital. Heart of balochistan coalfield. Mach is found among the stony mounts.

Sample collection;

A total of 150 samples were collected randomly from different areas of maach coalmines, during April 2017 to June 2017. After collection the samples were kept in thermopile box filled with crushed ice and were transported to bacteriology laboratory, CASVAB, University of Balochistan for further processing. All samples were processed within 4-5 hours of collection.

Fig:1

Fig: 2

Fig:3

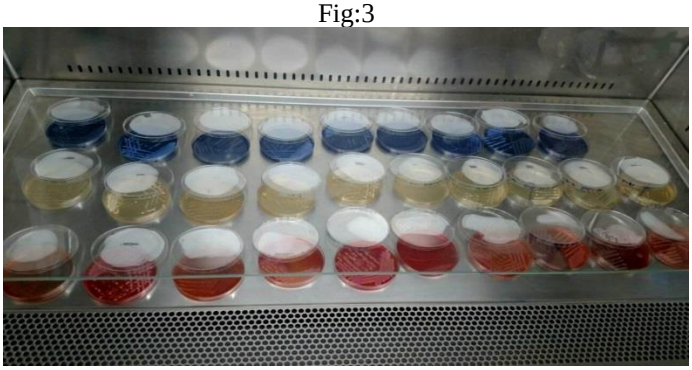


Fig:4

Microbiological analysis;

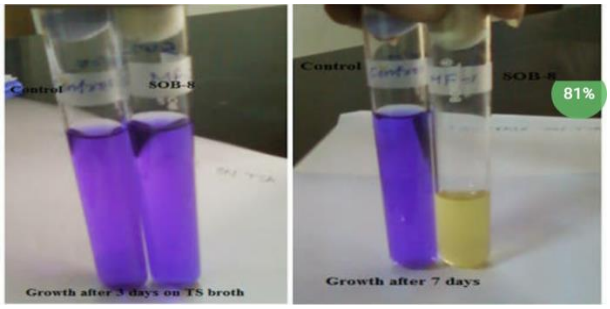
By using nutrient agar medium and serial dilution method microbiological study were performed. Microbial colonies were enumerated as colony forming unit (CFU). Viable cells will be considered from the plates after incubation at 37°C in a BOD incubator.

Subculture;

It was thru by streak plate manner taking the secluded colonies of bacterial cultures which were got from spread plate manner and again incubated at 37°C for 24-48 hrs.

Isolation;

Mines sewerage water, coal dust and sputum samples where processed for isolation of coal mine microbial flora. Take 2 to 5ml of sample in laminar flue hood cabinet following the sterility procedure. And transfer all samples in BHI(brain heart infusion) broth base media for enrichment. And incubate these all sample in incubator for 24 hour under 37°C.after 24 hours we check the growth in broth all tubes are turbid with bacterial colonies. Then we put these tubes in laminar flow hood safety cabinet for shifting purpose. Then we transfer these samples on agar base media. And inoculate these samples on makongey and nutrient agar plates. These agar are used as a primary ager for the isolation of bacteria. Makongey act as a selective for gram negative as well as a differential media. Then incubate these plates in incubator for 24 hours in 37°C.afters that we witness the growth. Colonies are well developed and clear. Then we use series of different agar and broth base media for selective isolation of bacteria. Theses media are iron sulphite nutrient media and broth, thiosulphate ager and broth(identification of sulphur oxidising bacteria),crystal violet inclusion sorbitol media, methylene blue sorbitol media, and salmonella and shigella ager for the isolation of salmonella, shigella and proteous etc. These ager and broth are incubate in different temperature according to the type of bacteria.



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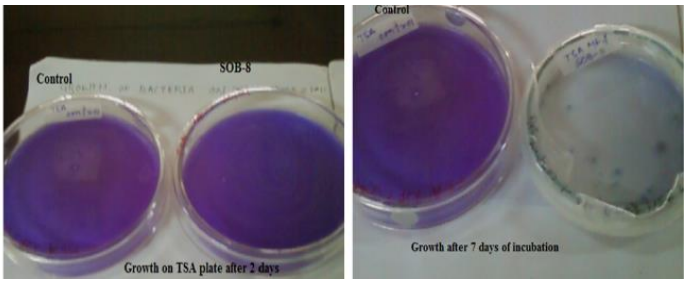

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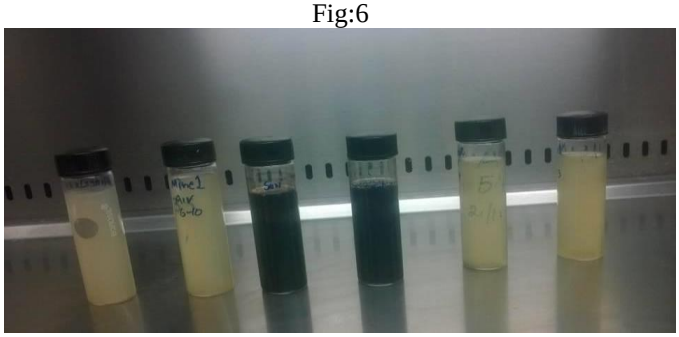

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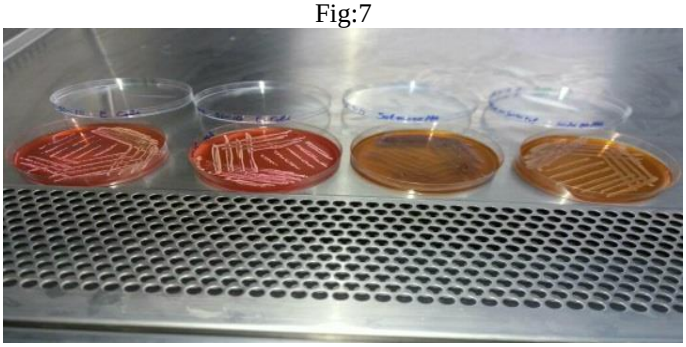

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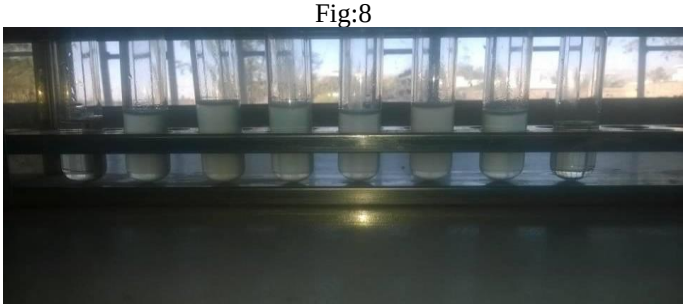

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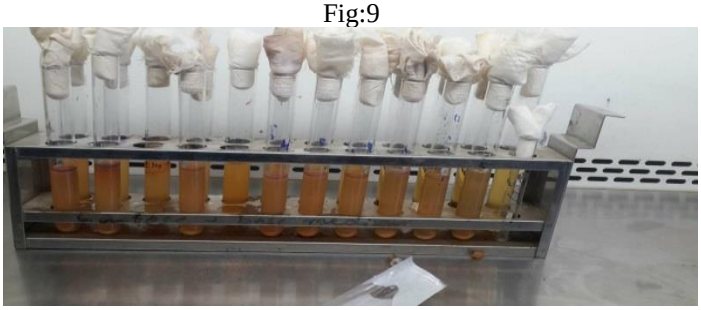


Fig:10

Fig:11

Microscopic identification of bacteria's:

Laboratory techniques microscopy the samples where processed for colony documentation. Direct wet fislm preparation were done for each sample at the same time.using normal saline(0.85./.) for detecting the actively motile organisms. All samples were examined microscopically by using 100x and 40xpowerlenses.the microscopic examination was done 3 times on each sample for confirmation. We observe almost all forms of bacteria like coccus, diplococcci, streptococci, sarcina , tetrad ,bacillus ,diplobacilli, streptobacilli, spirochete etc.

Literature Review;

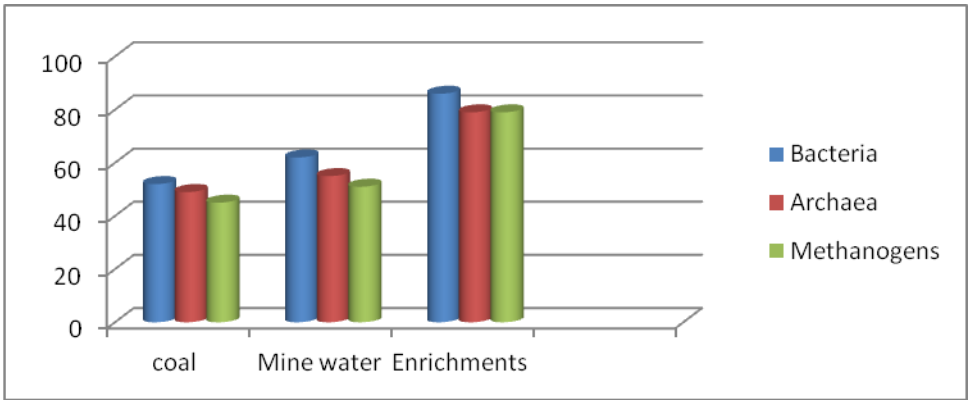
Microcosm using formation water as medium as well as a source of inoculum and coal as carbon source produced substantial quantity of methane which amplified considerably by the count of nitrite. The supremacy of Diaphorobacter sp. in nitrite modified microorganism showed their vital part in associate methanogenesis in the coal bed. This is the leading study demonstrating presence of methanogenic and bacterial community in an Indian coal bed that is accomplished of in situ biotransformation of coal into methane.(9) . A momentous mass of bacteria closely associated to the genus Syntrophomonas was identified at high dilution rates. Dilution rate showed an apparent effect on archaeal and bacterial populations in the butyrate-fed chemostats.(10) The two fens had actual comparable range of methanogenic methyl-coenzyme M reductase gene (mcrA), but in the superior film of the bog the methanogen range was amazingly lower, and merely one type of mcrA sequence was reclaimed. It was associated to the Fen mass, a group of unique methanogenic sequences establish formerly in Finnish mires. Bacterial 16S rDNA sequences from the fens fell into at most nine phyla, but merely four phyla were recovered from the bog. The furthermost common bacterial groups were Deltaproteobacteria, Verrucomicrobia and Acidobacteria.(11) These consequences advocated that past and on-going biodegradation of coal by methylotrophic methanogens and syntrophic bacteria, as well as thermogenic CBM fabrication, underwrote to the Liulin CBM reserves connected with the Eastern Ordos Basin.(12) The supremacy of Diaphorobacter sp. in nitrite amended microorganism shown their important part in supporting methanogenesis in the coal bed. This is the leading study indicating presence of methanogenic and bacterial community in an Indian coal bed that is capable of in situ biotransformation of coal into methane.(13) it demonstrates the resemblances and metamorphoses between the two environs with specific examples, from the flora of the organic molecules to the methanogenic metabolic alleyways and the construction of the microbial populations to demonstrate that widely diverging microbial populations show amazingly alike metabolic abilities.(14) . In contrast to acknowledged methanogenesis pathways containing one- and two-carbon compounds, this "methoxydotrophic" approach of methanogenesis couples O-demethylation, CO2 reduction, and perhaps acetyl-coenzyme A metabolism. Since MACs derivative after lignin might occur broadly in subsurface sediments, methoxydotrophic methanogenesis would play an important character in the creation of natural gas not limited to coal-bed methane and in the universal carbon cycle.(15) , the formation of [(13)C]methane was linked to acetoclastic methanogenesis in both the [(13)C]acetate- and the H(2)-(13)CO(2)-amended values of coal and timber. H(2)-(13)CO(2) was castoff mainly by acetogens related to Pelobacter acetylenicus and Clostridium species. Dynamic methanogens, closely associated with Methanosarcina barkeri, consumed the freely existing acetate rather than the thermodynamically more favorable hydrogen. Thus, the methanogenic microbial community seems to be extremely modified to the low-H(2) circumstances set up in coal mines. (16) the bacterial isolate along with the interfering of metal ion deliberation and substrate utilization. Cr6+ removal was found projecting even in bimetallic clarifications. The bacterial isolate was established to be Rhodococcus erythopolis by 16s rRNA molecular categorization. Hence the bacterial

isolate found from the coal mine area verified to be a impending agent for microbial remediation of Cr6+ laden excess water. (17) The personal property of the different inoculum was the same anyway of salt source (sodium bicarbonate, sodium chloride and artificial sea salt). As a final point, the microorganism-mediated leaf litter analysis was most well-organized at intermediate salinity levels (≈500µS/cm). The current study thus points to simple consequences of increasing salinity strengths on the ecosystem purpose of leaf litter breakdown, while the primary processes need more scrutiny.(18) The competences of oxidizing 10 g · L(-1) S(0) and 10 g · L(-1) pyrite were about 9.6% and 20%, respectively. Cells cultured in pyrite as substrate secreted more extracellular polymeric substances than they did when cultured in Fe(2+) or S(0). Furthermore, 75% total sulfur elimination and 86% pyritic sulfur elimination was attained in a sequencing batch reactor of bio desulfurization of coal.(19) . The sulphur relative contents analysis from XANES showed that the elemental sulphur (28.32%) and jarosite (18.99%) were accumulated in the bio treated remaining coal. However, XRD and XANES ranges of remaining pyrite shown that the sulphur mechanisms were mostly composed of pyrite (49.34%) and elemental sulphur (50.72%) but no other sulphur substances remained noticed. Based on the present results, we speculated that the pyrite forms in coal might affect sulphur bio oxidation procedure.

RESULTS:

In this study, samples were isolated from soil of Bolan District in the Balochistan province of Pakistan. A total of 150 samples were examined. 50 of coal dust samples,50 samples of airborne debris, Moreover 50 sputum mine worker samples. During this study we isolate various different groups of bacteria's. By the using of various growth media both agar and broth base. Series of various media contains different type of nutrients according to various bacterial groups. These media which we use in this study are BHI (brain heart infusion), nutrient, makongey, iron sulphite nutrient media, thiosulphate ager, sulphur oxidising, crystal violet inclusion sorbitol media,

Methylene blue sorbitol media, salmonella and shigella ager, Yeast Mannitol Agar medium, MSA, Nitrate Medium etc. And furthermore biochemical tests are also very important for the identification of some bacterial species. We harbour a number of microbes including harmful, non-harmful gram positive, gram negative, acidophilic, alkaliphilic, halophiles, and thermophiles within itself. We isolate acidophilic, heterotrophic, aerobic , motile, Gram negative bacteria are generally found. Acidophiles like Acidiothiobacillus sp. is major among them. Beside it, thermophilic bacteria like Thermoplasmatales sp. , rod shaped Pseudomonas sp. are significant . we also observe the colonies of Gram positive bacteria like Bacillus sp. , chemoautotroph like Ferrobacillus sp. , Flavobacterium acidurans in acid coal mine water , even archaea can be present. Acidithiobacillus ferrooxidans , Acidithiobacillus thiooxidans, Rhizobium.we also isolate organisms we are not coal mine representative organisms but always presents in every type of aerobic environment like Escherichia coli, salmonella and shigella, Proteus, Staphylococcal aureus, The percentage of bacteria's which we isolate from coal mines of mach are given to the below fingers and tables.



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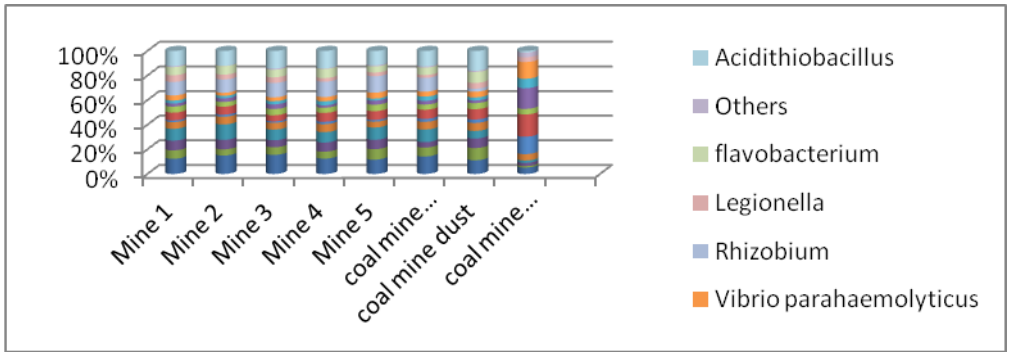


Fig:13

Bio chemical characteristics of coal mine microbial flora(Bacteria's)

Tests	Characteristics observed
Indole formation	-
Nitrate reduction	-
Fermentation of sucrose	+
Fructose	+
Catalase test	+
Oxidase test	+
Production of H_2S	+
Gelatine liquefaction	+
Di-Glucose	+

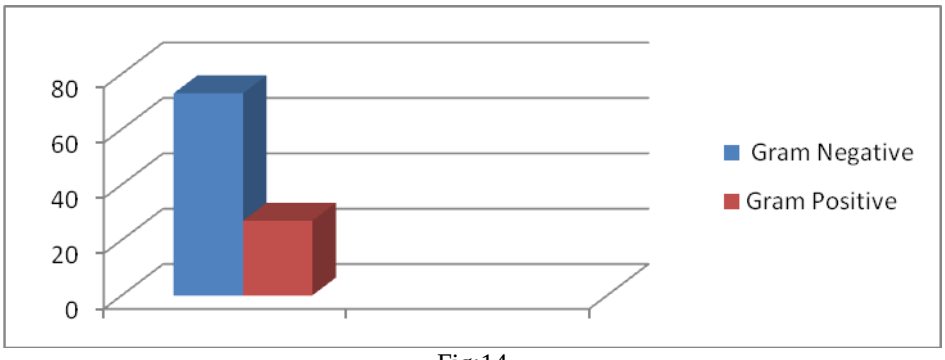
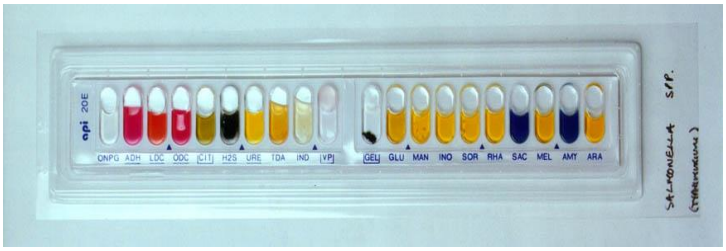


Fig:14

In physiological trials, the isolate was positive in the catalase and oxidate tests. Broad consequences for the API 20E test strip are scheduled in Table 1. The ONPG trial (tests for β-galactosidase enzyme) was lacking due to an unfixable air bubble that stalled results, and is not included.



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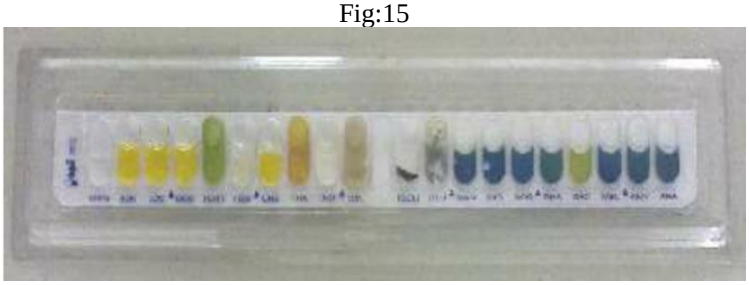

Fig:16

Fig:17

Fig:18

TABLE 1: A complete listing of the results from my API 20E test strip for my isolate.

Test	Result
CIT- senses consumption of citrate as merely carbon source.	Negative
TDA- exposure of the enzyme tryptophan deaminase	Positive
URE-senses of enzyme urease	Positive
LDC- regulates decarboxylations of the amino acid lysine through lysine decarboxylase	Negative
IND- recognition of production of indole by the enzyme tryptophanase	Positive
H2S- senses creation of hydrogen sulfide	Positive

VP- regulates if fermentation of glucose by bacteria consuming the butylene glycol pathway is being consumed.	Positive
INO- notices fermentation of inositol (cyclic polyalcohol)	Positive
SAC- senses fermentation of sucrose (disaccharide)	Positive
RHA- notices fermentation of rhamnose (methyl pentose sugar)	Positive
SOR- notices fermentation of sorbitol (alcohol sugar)	Positive
MEL- notices fermentation of melibiose (disaccharide)	Negative
ARA- senses fermentation of arabinose (pentose sugar).	Negative
AMY- senses fermentation of amygdalin (glycoside).	Negative
GLU- senses fermentation of glucose (hexose sugar).	Positive
GEL- trials for the production of the enzyme gelatinase.	Negative
MAN- senses fermentation of mannose (hexose sugar)	Positive
ODC- regulates decarboxylations of the amino acid ornithine through ornithine decarboxylase.	Negative
ADH- regulates decarboxylation of the amino acid arginine through arginine hydrolase.	Negative
LDC- regulates decarboxylations of the amino acid lysine through lysine decarboxylase.	Negative

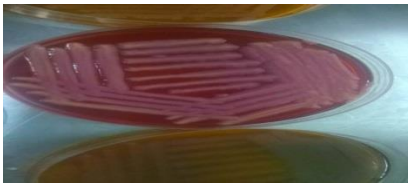

Fig:19

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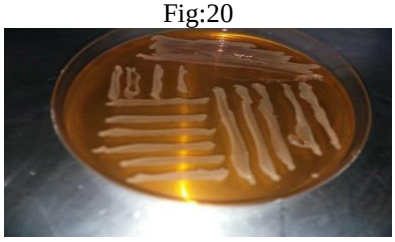

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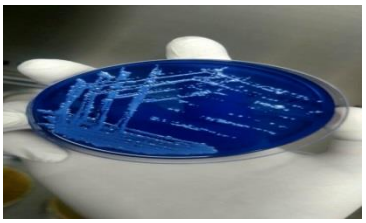

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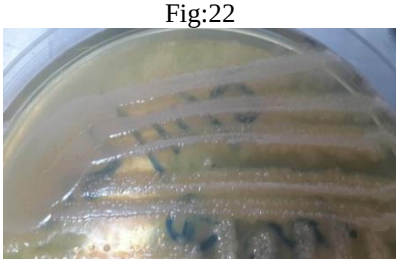

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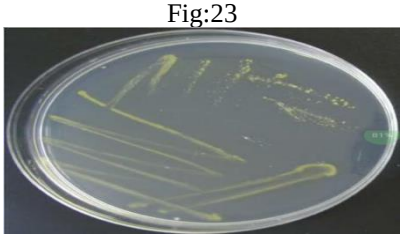

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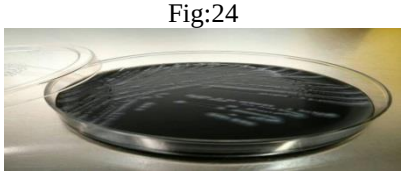

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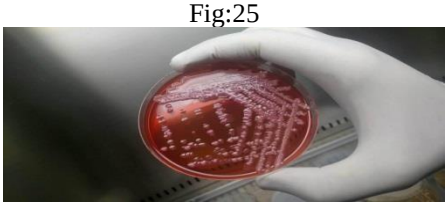

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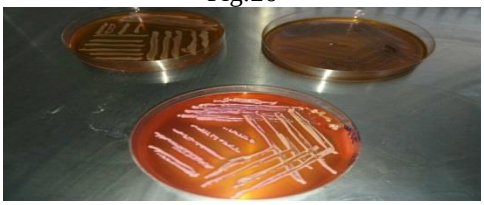

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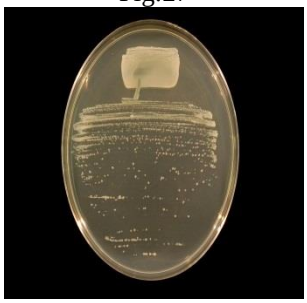

Fig:28

DISCUSSION:

Acid mine drainage (AMD) remains tremendously acidic, sulphate-rich effluent from wild or active mine spots that similarly hold preeminent stages of heavy metals. Raw AMD can foul surface and groundwater and pose Spartan ecological threat. .(20) The unseating of elemental sulphur, jarosite and ferric sulphate triggers in the third phase condensed the pyrite disposal and ferric iron concentration in the leachate and brought the procedure of bio desulphurization to an end.(21) Thiobacillus ferrooxidans are the leading iron- oxidizing bacteria in many viable processes for the bio oxidation of pyrite and connected ores.(22) The Gram-negative iron-oxidizing bacterium Leptospirillum ferrooxidans holds all genes needed aimed at nitrogen fixation, from genes encoding the Mo-Fe nitrogenase, the definite controller (nifA), global controllers like glnB and ntrC like genes, to further feelers and conveyance arrangements someway connected to nitrogen assimilation. . In our study we isolate different Species of coal mine microbial flora out of 150 samples from mach coal mines. during a period of 3 months April to June 2017. Similar findings were noted in a study carried out earlier.(23) The isolate did not appear to use the energy from ferrous iron oxidation. Both iron (ferrous or ferric) and an animate substrate remained compulsory to encourage growth. The separate displayed a minor tolerance to heavy metals than other iron-oxidizing acidophiles, and development was reserved by contact to light. There was confirmation of extracellular sheath construction by the isolate. In this and certain other compliments, the isolate be similar to members of the Sphaerotilus-Leptothrix group of filamentous bacteria. The guanine-plus-cytosine satisfied of the isolate was 62 mol%, which is less than that verified for Sphaerotilus-Leptothrix spp. and more than those of L. ferrooxidans and most T. ferrooxidans isolates.our study Also observe this phenomena of iron-oxidizing acidophiles. Acid H2O from a sealed deep coal mine was studied for the occurrence of heterotrophic bacteria. This H2O, which is regularly propelled to a outward stream for runoff, did not hold “acid streamers” or slime-producing, spore forming bacteria. But, aerobic, non-motile, yellow, non-fermentative, gram-negative rods remained originate by consuming the most-probable-number method with a dilute tryptone-yeast extract medium. The isolates displayed binary fission as the manner of replica although abortive partition, as verified by mini-cell creation, happened at a low rate. Based on morphological and additional characteristics, as well as deoxyribonucleic acid base configuration, a novel species, Flavobacterium acidurans, is projected for these bacteria. This study illustrated 30./. flavobaterium which always present in environment.

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