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FINAL REPORT

PROJECT N° 364-1486

"NATURALLY OCURRING PLASMIDS AND HEAVY METAL RESISTANCE OF *THIOBACILLUS FERROOXIDANS*"

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Naturally ocurring plasmids and heavy metal resistance of *Thiobacillus ferrooxidans*.

This is the final report from the Project "Naturally ocurring plasmids and heavy metal resistance of *Thiobacillus ferrooxidans*". It convers the total research period betwenn September 1984 to April 1987. The results are the following:

1. Standarization of the techniques for monitoring ferrous iron oxidation by *Thiobacillus ferrooxidans*.

   In relation to the techniques to be used in the project, the oxidoreduction and complexometric techniques in order to determinate Fe^{++}, Fe^{+++} and total Fe are standarizated.

   These techniques are used in order to carry out the quantification of iron in the kinetic studies of the chemical and bacterial oxidation of Fe^{++} to Fe^{+++}.

   When we make the ferrous determination using oxido-reduction technique with $KMnO_4$ like valorizer, we find that this technique doesn't have the sensibility that we need when the iron concentration is low (10 mg/ml). On the other hand when we use the complexometric technique with EDTA-Na like valoraizer and salicilic acid or xylenol orange like indicator, we find a very good sensibility, working at low concentration of iron. These results are shown in table 1 and fig. 1 and 2.

Fe+++ Determination

Complexometric Technique

Valorizer: EDTA-Na Indicator: Xylenol Orange

A blue complexo of Fe^{+++} - Xylenol Orange is formated and by titulation with EDTA-Na Solution cause a change of the blue color and a yelow color appear.

In order to determinate total Fe, is necessary to oxidize the sample with 3% H_2O_2 and make the total Fe valuation like Fe^{+++}.

Oxidation of Fe^{++} to Fe^{+++}

Bacterial Oxidation Process

Complexometric Technique

Kelly medium

TABLE 1 : OXIDATION OF FERROUS IRON MONITORED BY COMPLEXOMETRIC TECHNIQUE

Time days	EDTA (ml.) Exhausted volume	Fe^{+++} mg.
0	0.3	0.1680696
1	0.35	0.1694701
3	0.6	0.3641508
4	1.0	0.6022494
5	2.5	1.4005800
6	6.6	3.7253248
7	11.2	6.2745984

2. Isolations of Thiobacillus ferrooxidans and other bacteria water samples were collected from drainage at 27 mines. The mining zones are listed in Table 2. All samples were transported to the laboratory on ice.

Water samples were inoculated in a) TK medium (Tuovinen-Kelly 1973) for isolation of Thiobacillus ferrooxidans. This media has the following composition (g/lt): K_2HPO_4, 0.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5 g, H_2SO_4 0.11 N, 1,000 ml and $FeSO_4 \cdot 7H_2O$, 33.

TABLE 2 : MINING ZONES VISITED

Mining zones	Water samples
Junín	Coricancha
Lima	El Barón Aurelio Tamboraque
Arequipa	Cerro Verde Santa Rosa El madrigal Yura La Calera
Cerro de Pasco	Cerro de Pasco
Cajamarca	Tragadero Perolitos Socavón Real San Agustín Barragán Arpon Yola Los Mantos Mesa de Plata Pozos Ricos Carolina Desconocida
Huaraz	Caridad Coturcan Hércules Collaracra Monterrey

TABLE 3: MICROORGANISMS ISOLATED FROM WATER SAMPLE

Water sample	Medium			
	TK	TS6	TH	Luria
Coricancha	+	+	-	+
El Barón	-	-	-	+
Aurelio	-	-	-	+
Tamboraque	+	+	-	+
Cerro Verde	+	-	+	+
Santa Rosa	+	-	-	-
El Madrigal	+	-	-	+
Yura	+	-	-	+
La Calera	+	-	-	+
Cerro de Pasco	+	-	+	-
Tragadero	-	-	-	-
Perolitos	-	-	-	-
Socavón Real	+	-	-	+
San Agustín	+	-	-	+
Barragán	+	-	-	+
Arpón	+	+	-	-
Yola	+	-	-	+
Los Mantos	+	-	-	+
Mesa de Plata	+	-	-	+
Pozos Ricos	+	-	-	+
Carolina	+	-	-	+
Desconocida	+	-	-	+
Caridad	+	-	-	+
Coturcan	-	-	-	+
Hércules	+	-	-	+
Collaracra	+	-	-	+
Monterrey	-	-	-	-

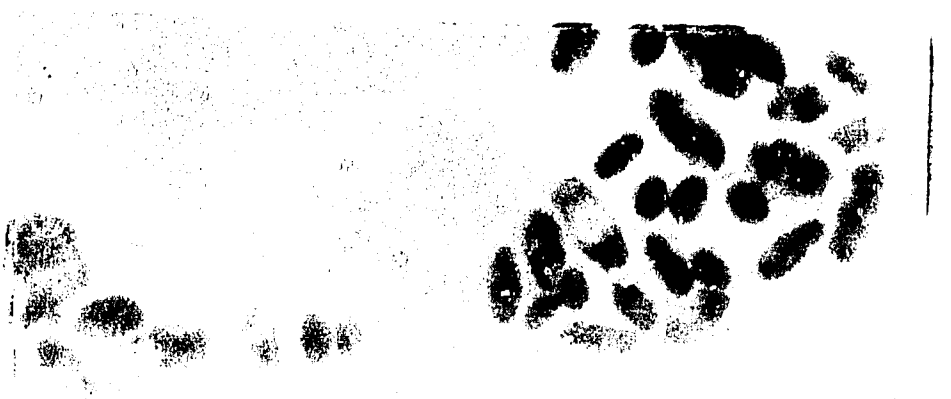
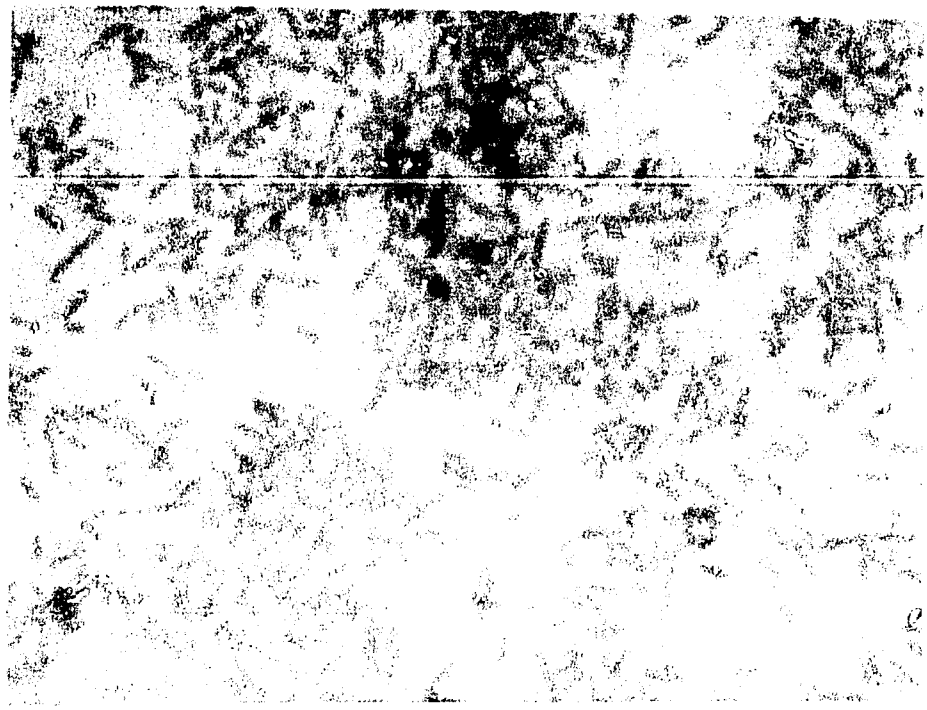
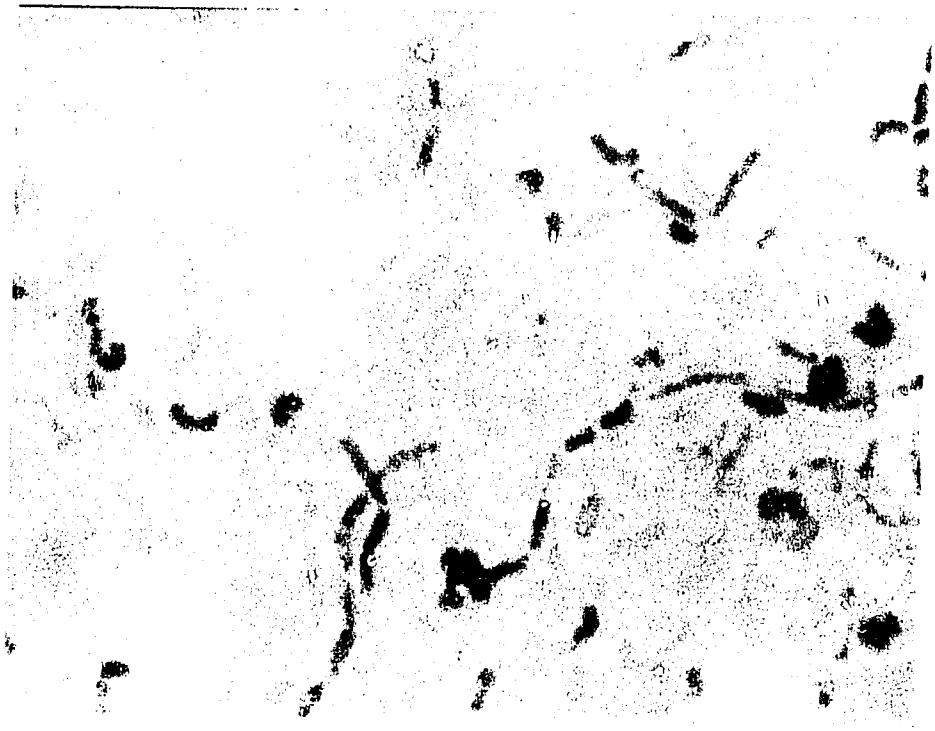


Fig. 3, 4, 5 and 6: Heterotrophs isolated from mining waters.





b) TS6 medium for isolation of Acidophilium cryptum. This media has the following composition (g/l): $(NH_4)_2 SO_4$, 0.2; Kcl, 0.01; K_2HPO_4, 0.025; Ca $(NO_3)_2$, 0.1; glucose 1% and 0.01% yeast extract.

c) TH medium, for isolation of thermophiles strains. This media has the following compositon (g/l): K_2HPO_4, 0.5; $(NH_4)_2 SO_4$, 0.5; Mg $SO_4 . 7H_2O$, 0.5; $FeSO_4 . 7H_2O$, 30; 0.02% yeast extract and H_2SO_4 0.11 N, 1000 ml.

d) Luria medium, for isolation of heterotrophs. This media has the following composition (g6L): Bacto triptone, 10; yeast extract, 5 and Na cl, 5.

It were isolated a high number of strains listed in Table 3. There were some hetetrophs which were present in differents mines water. Fig. 3, 4, 5 and 6 shows the morphology of these microorganisms wich can have some importance in the thiobacilli natural environment. Actuallly we are beginning to study if there is some king of relationship between these microorganisms and Thiobacillus ferrooxidans, but at this time we do not have results to show here.

3. Purification of Thiobacillus ferrooxidans.

To confirm the purity of T. ferrooxidans strains it was neccesary to grow these strains in solid media. We developed a highly efficient solid media which have the following composition (g/l): $(NH_4)_2 SO_4$, 3.0; Mg $SO_4 . 7H_2)$, 0.5; Kcl, 0.1; Ca $(NO_3)_2$, 0.018; $K_2 HPO_4$, 0.05; Fe $SO_4 . 7H_2O$, 4.4 g; Agar Oxoid L28, 7; and destilled water 1000 ml. The photograph 5 show colonies of T. ferrooxidans on solid media. We inoculate one colony in liquid medium. This procedure needed to be repeated at least three time. After third passage in liquid media, these strains were completely purified through resistance to silver. They were grown in Tuovinen-Kelley Medium with increasing concentrations of silver nitrate until they can not oxidize ferrous iron.

After we search for acidophiles presence in cultures of T. ferrooxidans: T. acidophilus and Acidophilum cryptum (the pattern strains were a generous gift of Dr. Harrison, University of Missouri). The media used for the isolation of A. cryptum contains the following (% w/v): 0.2 $(NH4)_2 SO_4$, 0.01 KCl, 0.025 K_2HPO_4, 0.025 $MgSO_4$ $7H_2$), 0.01 Ca $(NO_3)_2$, 0.1 % glucose and 0.01% yeast extract. T. acidophilus was looked for using Basal 9K medium of silverman and Lundgren with 1% glucose and 10 p.p.m. of ferrous sulfate.

With the techniques used we have demostrated that our cultures are purified and we got strains CH5 (Tamboraque, Lima) CH6 (Aurelio, Lima), CH18 (Santa Rosa, Arequipa)? CH40 (Cerro de Pasco, Cerro de Pasco), CH42 (Carolina, Cajamarca) and CH43 (Hércules, Huaraz).

We are continuing with the purification of the other strains; but the following experiments and results were accomplished with these six pure strains.

4. Studies of the physiology and genetics of pure strains CH5, CH6, CH18, CH40, CH42 and CH 43.

A. Utilization of sulfur compounds as energy source T. ferrooxidans strains CH5, CH6, CH18, CH40, CH42 and CH43 were inoculated in sulfur, thiosulfate and pyrite media. Sulfur medium were prepared by adding 0.5 % powdered sulfur (W/v) to basal media adjusted to pH 4.2 0 01% thymol blue was used as pH indicator.

Growth of the bacteria in sulfur, thiosulfate and pyrite media were detected by microscopic assay and verified by pH measurement. The sulfuric acid formed from the oxidation of sulfur, thiosulfate and pyrite caused lowering of pH concomitant with growth.

The results of growth in thiosulfate and sulfur are in the Table 4.

TABLE 4 : GROWTH OF *T. FERROOXIDANS* IN THIOSULFATE PYRITE AND SULFUR.

Strain	Sulfur Initial pH	Sulfur final pH	Energy Source Thiosulfate Initial pH	Energy Source Thiosulfate final pH	Pyrite Initial pH	Pyrite final pH
CH5	4.2	2.1	4.3	5.0	2.8	1.6
CH6	4.2	2.2	4.3	2.1	2.8	1.6
CH18	4.2	2.1	4.2	2.1	2.8	1.6
CH40	4.2	2.1	4.3	2.1	2.8	1.6
CH42	4.2	2.1	4.3	2.1	2.8	1.6
CH43	4.2	2.2	4.3	2.1	2.8	1.6

TABLE 5 : EFFECT OF INITIAL INOCULUM IN THIOSULFATE UTILIZATION

Cepa	Inoculum	Growth on Thiosulfate
CH5	10^6 bact/ml	+--
	10^7 bact/ml	+++ slow

TABLE 6: ABILITY OF USE THIOSULFATE AS ENERGY SOURCE

CH5	Growth on Thiosulfate		Total
# col	10	140	150
%	7	93	100

TABLE 7 : USE OF THIOSULFATE BY REPURIFIED CH5 STRAINS

Strain	Thiosulfate	pH Initial	pH final
CH5	tio +	4.0	2.1
EL-77	t	4.0	3.5
EL-78	t	4.0	3.5
EL-79	t	4.0	3.6
EL-85	t	4.0	3.5
EL-89	t	4.0	3.4
EL-100	t	4.0	3.7
EL-61	tio^-	4.0	6.0
EL-75	tio^-	4.0	6.1
EL-111	tio^-	4.0	5.8

TABLE 8 : UTILIZATION OF SULFUR AND FERRIC COMPOUNDS BY *T. FERROOXIDANS* TIO^-

Strain	Fe^{2+}	S°	S_2O_3	SFe
CH5	+	+	+	+
EL-61	+	+	-	+
EL-75	+	+	-	+
EL-111	+	+	-	+

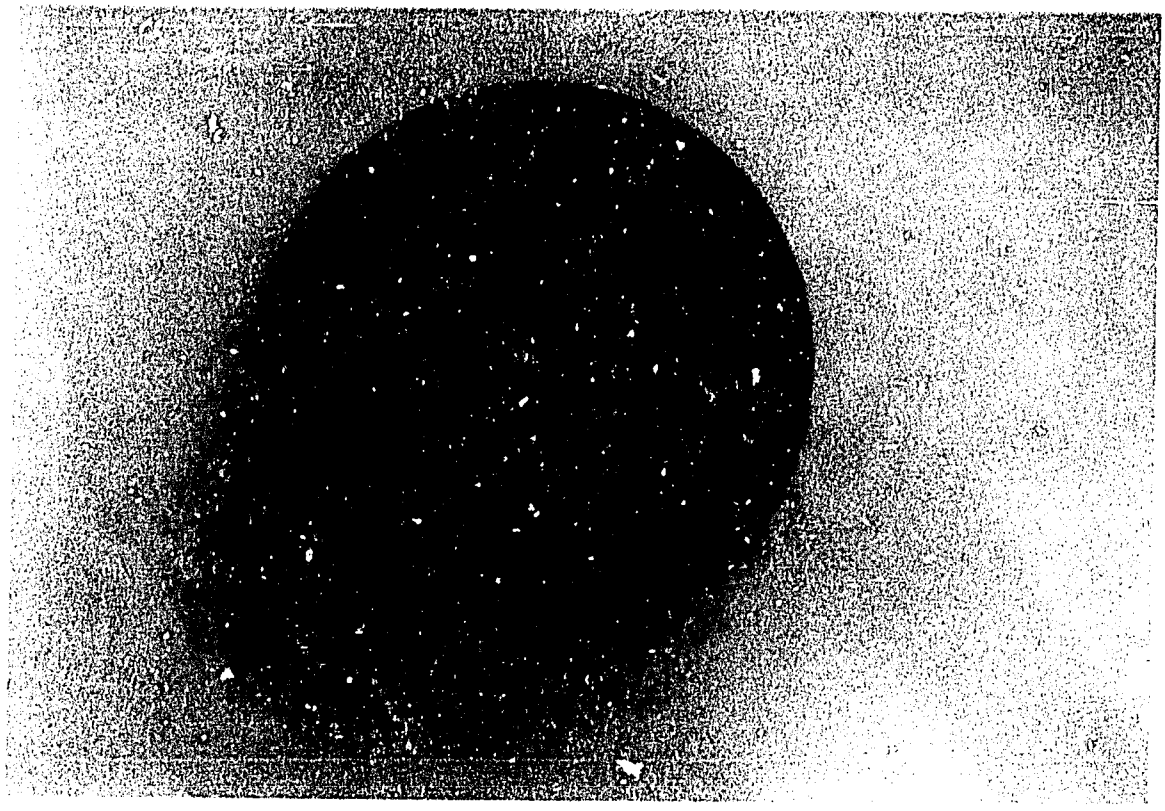


Fig. 7: Growth of T. ferrooxidans in solid media.

CH 5 had an odd growth in thiosulfate when was used an inoculum of 10^6 bact/ml these were no growth. With a 10^7 bact/ml these were no growth. With a 10^7 bact/ml inoculum there were slight growth (Table 5). We thought the posibility to have a mixed population of Thiobacillus ferrooxidans, one of then can grow in thiosulfate and another can not. In order to prove that, we screened 150 clones of CH5 (purified by growth in solid media) on their ability to use thiosulfate as energy source. The results are in Table 6.

These 10 colonies were screened again, giving them the best conditions to use thiosulfate: metal ions supplement, very high inoculum and longer times of incubation. With these conditions, we obtained six strains thiosulfate deficient (t) and three strains were incapable to use thiosulfate as energy source (tio^-) (Table 7).

Thiobacillus ferrooxidansEL-61, EL-75 and EL-111 were screened by their capability of sulfur and ferric compounds utilization (Table 8), but they shown the same pattern of growth.

We have developed a thiosulfate solid media to use thiosulfate as a marker in T. ferrooxidans genetic studies. And finnally, we are begining electrophoretic whole-cell patterns and 6-C contents with EL-61, EL-75 and EL-111 to get a better knowledge of these natural mutants.

B. Organic compounds inhibition.

Organic compounds inhibition were assesed by examining growth and oxidation of ferrous sulfate in Tuovinen-Kelley (T-K) medium with different concentrations of sodium piruvate, sodium citrate, lactose and glucose.

The profile of inhibition in T. ferrooxidans.

Strains CH5, CH6, CH18, CH40, CH42 and CH43 are in Table 9.

Strain	Lactose	MIC Sodium Citrate	Sodium Piruvate	Lactose	Glucose
CH5	10	10	0.35	0.10	NI
CH6	10	10	0.62	NT	NI
CH18	10	10	0.10	0.10	NI
CH40	5	NT	0.55	NT	NI
CH42	NT	NT	NT	NT	NI
CH43	NT	NT	NT	NT	NI

TABLE 9 : IRON OXIDATION INHIBITION BY ORGANIC COMPOUNDS ND: NO DATA, NI: NO INHIBITION (MAXIMUN CONCENTRATION TESTED: 100 mM).

There were a big difference between our results and from another laboratories because there were not inhibition of ferrous iron oxidation using glucose as organic compound added to T-K medium. We decided to test if our strains are capable to utilize that glucose as carbon or energy source. In order to try that we grow CH18 in TK mediun with ferrous and glucose and we monitored initial and final glucose, initial and final ferric iron and initial and final cell concentration.

The results are giving in Table 10. They show that there is not utilization of glucosa, our strains only use ferrous iron as energy source and CO_2 as carbon source, but there is no inhibition by presence of glucose in TK medium.

C. Metal Resistance.

Metal resistance were assesed by examining growth and oxidation of ferrous sulfate in TK medium with a range of metal concentrations. The metals used were Antimony (Sb) as $C_4H_4KO_7Sb$, Arsenic (As) as Na_3AsO_4, Cadmium (Cd) as $CdCl_2 . H_2O$, Cobalt (Co) as $Co Cl_2 . H_2O$, Copper (Cu) as $SO_4Cu . 5H_2O$, Chromium (Cr) as $(NO_3)_3 Cr . 9H_2O$, Tin (Sn) as $Cl_2 Sn . 2H_2O$ Mercury (Hg) as $HgCl_2$, Nickel (ni) as $SO_4 Ni . 6H_2O$, Selenium (Se) as Se, Tellurium (Te) as $K_2 teO_3$, zinc (Zn) as $ZnSO_4 . 7H_2O$ and Molybdenum (Mo) as $(NH_4)_6 Mo_7 O_{24} . 4H_2O$.

CH 18	Substrate		glucose		ferric iron		cell number	
	glucose	ferrous	initial	final	initial	final	initial	final
+	–	+	0	0	0.54	6.84	3.42×10^{6}	1.67×10^{8}
+	+	+	1.06	0.965	0.54	6.76	3.81×10^{6}	1.70×10^{8}
+	+	–	1.049	0.925	0	0	4.85×16^{6}	7.10×10^{8}
–	+	–	0.860	0.925	0	0	0	0
–	+	+	1.005	1.022	0.54	1.10	0	0

TABLE 10 : GROWTH OF CH18 IN GLUCOSE AND FERROUS IRON.

The profiles of metal resistance in T. ferrooxidans strains CH5, CH6, CH18, CH40, CH42 and CH43 are in Table 11.

Metalic ion	Strain CH5	CH6	CH18	CH40	CH42	CH43
Ni	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M
Cu	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M
Zn	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M	$5x10^{-1}$M
Cd	$3x10^{-2}$M	$1x10^{-2}$M	$3x10^{-2}$M			
As	$7x10^{-2}$M	$7x10^{-2}$M	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M	$1x10^{-1}$M
Hg	$7x10^{-7}$M	$7x10^{-7}$M	$7x10^{-7}$M	$1x10^{-7}$M	$1x10^{-7}$M	$1x10^{-7}$M
Mo	$1x10^{-5}$M	$1x10^{-5}$M	$1x10^{-5}$M	$1x10^{-5}$M	$1x10^{-5}$M	$1x10^{-5}$M
Cr	$1x10^{-3}$M	$1x10^{-3}$M	$1x10^{-3}$M	$5x10^{-3}$M	$1x10^{-3}$M	$5x10^{-3}$M
Sn	$1x10^{-3}$M	$1x10^{-3}$M	$1x10^{-3}$M	$5x10^{-3}$M	$1x10^{-3}$M	$5x10^{-3}$M
Co	$1x10^{-2}$M	$1x10^{-2}$M	$1x10^{-2}$M	$5x10^{-1}$M	$1x10^{-1}$M	$5x10^{-1}$M

TABLE 11 : Metal resistance of T. ferrooxidans strains CH5, CH6, CH18, CH40, CH42 and CH43.

It was screened the effect of metal ions over different concentrations of feroous and sulfur as energy source. It was assesed ferrous iron concen - trations of 5g, 10 g, and 30 g. The results are in Table 12.

There are not major differences among the metal resistance of T. ferro - oxidans strains CH5, CH6, CH18, CH40, CH42, and CH43, but it exists slights differences in metal resistance by CH18 as it is shown in Table 12. The metal resistance level remains the same in all the strains even though the original mine environment was very different.

TABLE 12 : METAL RESISTANCE OF T. FERROOXIDANS.

CH5, CH6, and CH18 with different concentrations of ferrous iron and sulfur.

ION	Cepas	5 g Fe	10 g Fe	30 g Fe	5 g Azufre
Ni, Zn (M)	CH5, CH6, CH18	0.5	0.5	05.	0.1
Cu, Al (M)	CH5, CH6, CH18	0.3	0.3	0.3	0.1
Co (M)	CH5, CH6, CH18	0.3	0.3	0.5	0.02
Sb (mM)	CH5, CH6, CH18	10	5	5	5
Sn (mM)	CH5, CH6, CH18	1	1	1	0.1
Cr (mM)	CH5, CH6, CH18	1	5	1	1
Cd (mM)	CH5, CH6, CH18	50	50	50	50
As (mM)	CH5	50	10	70	5
	CH6	50	10	70	5
	CH18	50	50	100	10
Mo (uM)	CH5	10	10	10	10
	CH6	10	10	10	5
	CH18	10	10	10	5
Ag (uM)	CH5	3	1	1	5
	CH6	3	1	1	5
	CH18	1	1	1	10
Hg (uM)	CH5	0.7	0.7	0.7	5
	CH6	0.7	0.7	0.7	5
	CH18	0.7	0.7	0.7	10
Te (uM)	CH5	5	5	5	1
	CH6	5	5	5	1
	CH18	5	5	5	5

D. Nitrogen Compounds utilization.

It was assesed utilization of urea and no nitrogen source in TK medium by examining growth and ferrous iron oxidation.

The results of these experiments in T. ferrooxidans strains CH5, CH6 and CH18 are in Table 13.

Aminoacids inhibition were assesed by examining growth and oxidation of ferrous sulfate in Tuovinen-Kelly (T-K) medium with the following pools of aminoacids.

Pool	Aminoacids
1	histidine, phenylalanine, glutamine
2	leucine, tyrosine, asparagine, serine
3	isoleucine, tryptophan, glutamic acid
4	methionine, lysine, threonine, aspartic acid
5	thiamine, valine, arginine, glicine
6	methionine, thiamine
7	histidine, leucine, isoleucine, lysine, varine
8	phenilalanine, tyrosine, thriptophan, threonine
9	glutamine, asparagine, aspartic acid, arginine
10	serine, glutamie acid, DAD, glycine

Augment of lag period produced by inhibition are listed in Table 14. The results show that there is not strong inhibition in T. ferrooxidans CH5, CH6, CH18, CH42 and CH43. T. ferrooxidans CH40 was completely inhibited by pools 4 and 6. The only common aminoacid is methionine. Actually, we are testing methionine as inhibitory of ferrous iron oxidation. If we can confirm it methionine would be our second marker and we would managed a genetic system to improve T. ferrooxidans metal resistance level.

TABLE 13 : EFFECT OF GROWTH AND OXIDATION OF FERROUS SULFATE USING AMONIUM SULFATE (TK MEDIUM), UREA (U) AND NO NITROGEN SOURCE (-N).

Strain / Concentration (bact/ml)	CH18			CH6			CH5		
	TK	U	-N	TK	U	-N	TK	U	-N
Initial	1×10^6	1×10^6	1×10^6	2×10^6	2×10^6	2×10^6	3×10^6	2×10^6	2×10^6
Final	2×10^8	2×10^8	2×10^7	2×10^8	2×10^8	2×10^7	2×10^8	2×10^8	2×10^7

TABLE 14 : EFFECT OF AMINOACIDS ON GROWTH AND OXIDATION OF FERROUS SULFATE.

Nº d: Number of days of lag period, N 6: No growth.

Pool	Strain					
	CH5	CH6	CH10	CH40	CH42	CH43
Control	4d	3d	4d	5d	3d	3d
1	4d	3d	4d	5d	3d	3d
2	4d	3d	4d	6d	3d	3d
3	4d	4d	4d	6d	3d	3d
4	7d	4d	7d	ND	4d	3d
5	4d	3d	4d	5d	3d	3d
6	4d	3d	4d	ND	3d	3d
7	4d	3d	4d	5d	3d	3d
8	4d	3d	4d	5d	3d	3d
9	4d	3d	4d	9d	3d	3d
10	5d	3d	5d	5d	3d	3d

E. Plasmid Profils.

For plasmid isolations, batch cultures were grown in 1 liter flask. The bacteria were harvested and the pellet was washed in acidic water. The resulting pellet were resuspended in TES buffer (50mM Tris, 2mM EDTA, 25% sucrose, pH 8.0) to induce Sphecoplast formation, Lysis of the cells was accomplished by adding 15% SDS and Proteinase K (2mg ml^{-1}).

Sodium chloride 5M was added to a final concentration of 1 M and was held at 4º C overnight to precipitate chromosomal DNA and SDS protein comple - xes. Centrifugation at 35,000 xg for 40 min. at 4º C precipitated the ce - llular debris. The supernatant containing various plasmid forms were preci pitated with 2.5 volumes of 95% ethanol and glucogen (1 mg ml^{-1}). This mixture was held at -20 C overnight to aid in the precipitation of the nucleic acids followed by centrifugation. The pellet was resuspended in TE buffer and stop mix.

The covalentely closed circular forms of plasmid were resolved by 0.7% agarose gel electrophoresis (4h 30 mA) in TEA buffer (40mM Tris, 2 mM EDTA, 20 mM Sodium acetate, ph 8.05). Plasmids of known molecular weights were used as standars.

The sizes of plasmids of CH5, CH6, CH18, CH40, CH42 and CH43 are indicated in Table 15 and in fig 8.

TABLE 15 : PLASMID PROFILE IN *T. FERROOXIDANS* CH5, CH6, CH18, CH40, CH42 and CH43.

Strain	Plasmid designation	Plasmid size (x 10^{-6})
CH5	pCH-51	22
	pCH-52	6.5
CH6	pCH-61	22
CH18	pCH-181	22
	pCH-182	6.5
CH42	No plasmid	
CH40	pCH-401	22
CH43	pCH-431	22
	pCH-432	6.5

CH5, CH18 and CH40 apparently the same plasmid profile (but to conform that we are begining to do some restriccion enzimes work), CH12 have not plasmids and CH6 and CH42 have the same apparentyly plasmid profile also, but all of them have the same metal resistance level, organic compounds inhibition, sulfur compounds utilization and nitrogen compounds inhibition. There is not relationship between presence of plasmids and any studied character.

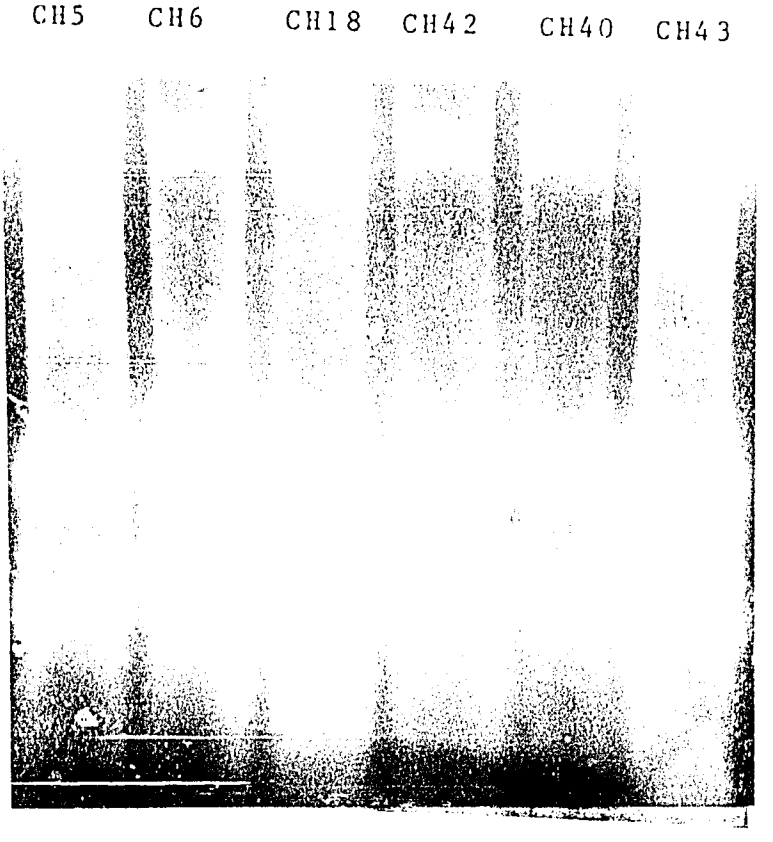


Fig. 8 : Plasmid screen in T. ferrooxidans CH5, CH6, CH18, CH40, CH42 and CH43.

CONCLUSIONS

1. It were isolated a high number of *Thiobacillus ferrooxidans* from different mineralogical zones containing high concentrations of metals. These strains have potencial genetic resistance to different mineral. It is possible to select one of them to be used in bacterial leaching in any special kind of peruvian minerals.

2. It was got six pure strains of *T. ferrooxidans* wich have been studied in their biochemical, phisiological and genetical characteristics, it has permited us to get a better knowledge of this specie and recognition of some inhibitory factors. These results will be applied in practical leaching work.

3. There are some results as alternatives sources of nitrogen and lack of inhibition by some organic compounds wich offer posibilities of inmediate aplication in bacterial leaching processes.

4. There is no relationship between metal resistance or any another characteristic and plasmid presence. Metal resistance level remains the same in all studied strains even though different mineral and metal concentration in bacteria original environment. Differences in metal resistance might be related to chromosomal DNA.

5. Has been developed a highly efficient solid media for *Thiobacillus ferrooxidans* and six pure strains well characterized. By using one of them CH5, it has been isolated three natural thiosulfate mutants: EL-61, EL-111 and EL-75. It has been demostrated that CH40 become inhibited by the presence of one aminoacid, there is a strong posibility that methionine will be the inhibitory aminoacid (we are finishing some research to confirm it). We have some plasmids bearing strains and CH42 wich has no plasmid in it. With all this results we are ready to begin genetic studies to improve metal resistance in *Thiobacillus ferrooxidans*.