REVISTA DE METALURGIA, 43 (3)
MAYO-JUNIO, 228-236, 2007
ISSN: 0034-8570

Biochemical analysis of the *Hormoconis resinae* fungal mycelium in the corrosion of aeronautical aluminium alloys*

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Abstract

Biochemical analyses of the *Hormoconis resinae* fungal mycelium would explain behaviour differences of corrosive and non-corrosive strains on Al and its aeronautical alloys. In previous works its aggressiveness had been studied through SEM-EDX surface analysis, electrochemical techniques and immersion testing. In this paper separation of the proteins of the mycelium produced by a non-corrosive strain and its culture along three generations was performed. Cultures were prepared in batch in the presence and absence of pure Al and AA 2024, AA 7005 and AA 7075 alloys. The mycelia grown throughout the three generations increasingly recovered usual characteristics at the third replication, included their corrosiveness on Al and its alloys previously shown by all our strains. Amongst the bio-molecule fractions isolated and analysed during this preliminary study only the proteins revealed changes with the generation grown. When this fungal strain was cultured in the presence of alloy metal sheets electrophoresis of the protean fraction was correlative with the distinct mycelia behaviour observed, including corrosiveness on Al and its alloys.

Keywords

Hormoconis resinae. MIC. Aeronautic. Aluminium alloys. Biochemical analyses.

Análisis bioquímico del micelio del hongo *Hormoconis resinae* en la corrosión de aleaciones aeronáuticas de aluminio

Resumen

Las diferencias entre el comportamiento corrosivo y no corrosivo de una cepa del hongo *Hormoconis resinae* sobre aluminio y sus aleaciones aeronáuticas se explicarían a través de análisis bioquímicos del micelio. En trabajos previos, el comportamiento corrosivo se estudió mediante análisis de superficie SEM-EDX, técnicas electroquímicas y ensayos de inmersión. En este trabajo, se llevó a cabo la separación de proteínas del micelio producido por una cepa que perdió su corrosividad y su cultivo a través de tres generaciones. Cultivos en *batch*, en presencia y ausencia de aluminio y sus aleaciones AA 2024, AA 7005 y AA 7075, a través de tres generaciones del micelio crecido, fueron recuperando sus características, incluida su habitual corrosividad, en la tercera replicación. De las fracciones de biomoléculas separadas y analizadas durante este estudio preliminar, sólo, las fracciones proteicas revelaron cambios de una a otra generación. Cuando esta cepa del hongo se cultivó en presencia de probetas de los metales, las modificaciones en la electroforesis de las respectivas fracciones proteicas fueron correlativas del comportamiento del micelio frente a la corrosión del aluminio y sus aleaciones.

Palabras clave

Hormoconis resinae. MIC. Aeronáutica. Aleaciones de aluminio. Análisis bioquímico.

* Trabajo recibido el día 21 de diciembre de 2005 y aceptado en su forma final el día 6 de marzo de 2007.
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1. INTRODUCTION

Several studies in the international literature have reported the effect of *Hormoconis resinae* in the corrosion of Al and its alloys. The mechanisms proposed to explain the microbiologically influenced corrosion (MIC) found have extended in the last decades to analysis of in-service failures associated to different aluminium alloys in aircraft integral fuel tanks[1-7]

Widespread techniques for these studies have been optical microscopy and scanning electron microscopy (SEM), together with several electrochemical techniques[7-15]. EDX (energy dispersive X-ray analysis) showed novel information on the existence of preferential sites of attack and provided earliest evidence on possible biological factors determining the location of such sites[10-16]. First stages of corrosion showed evidence of enhanced susceptibility of certain metal areas to localised attack, determined by alloy chemical heterogeneities with respect to that of the alloy matrix, designed "secondary phases"[11-16]. Such results would suggest that bio-molecules with different functional groups could be needed to produce such localised up-take attacks.

The stages of massive corrosion disguise these initially selective attacks, but complementary techniques were applied to investigate the possible existence of a biological driving force responsible for the phenomenon. To this purpose measurements of several metal-ion uptake yielded results of great interest to further advance in the hypotheses proposed as a basis for the present work. Similar orders of magnitude of such metal up-take for Fe, Zn, Al, Cu, Mg, and Na ions by the *Hormoconis resinae* fungus[17 and 18] were found to those exposed for bacteria in the numerous works on the subject in the revision work by Brown and Lester, 1979 [19]. This similarity suggests an interesting common pattern of behaviour for several microbial species in the presence of varied essential metal ions (oligoelements).

Should these interactions depend on a biological driving force, the MIC phenomenon could be clarified by knowing the effect of the presence or absence of essential metal ions on the biochemistry of microbial species. A valuable innovative tool would thus be explored for the design of MIC resistant alloys alternative to the extended use of biocides as considered the only available solution.

Such knowledge could drastically improve the established treatment of this problem, namely the use of biocides. These substances, of generally low specificity, besides inactivating corrosive microorganisms, should also affect other species, contributing to break the balance of the ecosystems they circulate alongside industrial waste, including different drinking water sources.

Consideration of oligoelements as essential nutrients is not only associated with the need of up-taking certain metal ions by microbial species but also, albeit unexpected, when its growth is conditioned to the presence of a piece of the respective metal. A first report of such phenomenon was briefly mentioned long time ago in a paper devoted to biocides evaluation[20] without further analysis of any possible explanation. It describes the difficulty to grow in laboratory microbial contaminants of sea-water used as ballast in water displaced ship-fuel tanks. However, the subject was not recognised as a valuable tool in the international literature until 1987 [10] with the description of a MIC problem at a steel-works plant. Failure occurrence was limited to a Sn-based babbitt antifriction coating of cold-steel roller mills, while Pb-based homologous antifriction coatings under the same whole set of operational conditions in the plant was not susceptible to MIC. These results suggest that the attack should not only be conditioned by the presence of microorganisms, nor by other chemicals available in the environment. It was found that it is also necessary that some of the alloy components be an "oligoelement" for the microbial contaminant/s. In fact the microbial growth of the isolated bacteria, *Pseudomonas maltophilia*, showed growth stimulation in the presence of Sn^{+2} salts while Pb ions did not promote its amount of growth.

Therefore, the aim of this work is to propose a "susceptibility" criterion allowing to detect "oligoelements" involved in an alloy formulation towards a given microbe. With this purpose biochemical analysis of the *Hormoconis resinae* fungal mycelium was approached, in the presence and in absence of Al and three of its aeronautical alloys having evidenced in the past very intense susceptiblility[7-18].

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Microorganisms and culture media

The strain applied to this study was one of various isolates from integral fuel tanks of military Argentine aircraft involved in a long duration study. After a four year storage period it showed unusual growth characteristics as loss of its dark coloration, lag phase duration increase, less amount and slower mycelium production, softer texture and lack of corrosiveness on aeronautical Al alloys.

To establish the possible cause for these variations this fungal strain[18] was grown as static batch cultures in a modified (m) [6] Bushnell-Haas (B-H) medium[21] (Table I) diluted to 1:10 solution with distilled water according to the conditions followed in previous works[6, 8, 9 and 11-18]. This diluted medium was used to support the fungal growth and to simulate the drainage water usually found

Table I. Bushnell and Haas Modified Nutrient Solution

Tabla I. Solución nutriente Bushnell y Haas Modificada

Compound	Weigth
$MgSO_4$ 7 H_2O	0.2 g
KH_2PO_4	1.0 g
$CaCl_2$	0.02 g
NH_4NO_3	1.0 g
Sol. 60% $FeCl_3$	2 drops
Distilled H_2O	1 l

Concentrated solution pH = 5.4.

as sediment in integral fuel tanks of in service aircraft. Its initial pH = 5.4 was adjusted to 6.5 and Jet A 1 fuel was used as the only carbon source. In military aviation this fungus appears since the 60´s as the most frequently reported corrosive microbial contaminant.

The fungal non-corrosive strain had been stored at -75 ºC temperature in the rich agar-malt medium during various years, and their corrosive behaviour, as well as other more obvious characteristics being recovered by successive replications[15]. The strain became re-adapted to grow in diluted mB-H medium and Jet A1 turbofuel as only carbon source at its third generation evidencing all its original characteristics, amongst them its corrosiveness.

2.1.2. Obtaining the homogenate

By re-suspension of a weighed biomass bulk of mycelium in a citrate-phosphate buffer, pH=5.0, the homogenate was obtained using physical methods of cellular rupture as ultrasound and maceration for its liquation.

2.1.3. Purification and characterisation of biologic macromolecules

An extraction of proteins present in the crude homogenate of the fungus was carried out using chromatographic methods and electrophoresis in polyacrylamide gels to establish the differences in protein composition of the various cellular extracts grown with and without Al-based alloys. Analogous preliminary characterisation work on the polysaccharide, lipidic and nucleic acid fractions separated from the grown mycelia did not seem to be influenced by the presence of metallic surfaces on the physiological response to the corrosive behaviour of this fungus on Al and its aeronautical alloys.

Both lipids and phospholipids were isolated from the crude homogenate for purification and characterisation through chromatographic methods in thin and gaseous layer. Carbohydrates were characterised using gaseous chromatography and mass spectrometry. As none of these latter bio-molecules revealed neither alteration amongst culture generation nor any effect of the presence of metals in the culture media, the biochemical further analyses were limited to the protean fraction.

The proteins present in the homogenate were precipitated by saline fractioning (with ammonium sulphate) and the fractions obtained subsequently dialysed against a buffer and afterwards concentrated. Separation of the precipitated and dialysed proteins was performed by chromatographic methods: a) molecular exclusion with Sephadex G-25, for isolation by molecular size and b) ionic interchange with DEAE-Sepharose, to separate proteins by batch[22]. The proteins thus separated were submitted to electrophoresis in polyacrylamide gels-SDS [23] to estimate molecular weight by comparing their electrophoretic mobility with already known standards.

2.1.4. Protein extraction

The treatment performed to obtain the protein homogenate was the following: the non-corrosive strain of the fungus was incubated at 34 °C in static batch during one month, after which the mycelium was extracted from the culture medium and washed with pH 7.0 phosphate citrate buffer. Following overnight digestion at 3 ºC, (digestion medium contained 0.5 % TWIN, 3.5 % SDS, 3 % glycerol, 1.0 mM EDTA, 1.5 mM $MgCl_2$ and 5.0 mM PMSF) the homogenate was centrifuged, the overfloating separated from the pellet, and proteins were determined by quantification in the crude extract performed by the Lowry et al method[24], using the Folin reactive and the Bradford method[25], based on adsorption of the Coomassie G-250 blue dye. In both methods the standard used was bovine serum albuminae (BSA).

Proteins characterisation present in the crude homogenate of the fungus estimated molecular weight by comparing their electrophoretic mobility. Thus differences in protein composition of the various cellular extracts grown with and without Al-base alloys was performed using electrophoresis in polyacrylamide gels (PAGE-SDS) at 10 %, dyed with coomassie blue and 30 µg protein was loaded into each gel track. The standard of molecular weights used contained Bovine sero albuminae (BSA) 66 kD; Egg albuminae 45 kD and Carbonic anhydrase of bovine erythrocytes 29 kD.

2.2. Metal samples

Al and AA2024, AA7005 and AA7075 alloys (Table II) of 10 x 10 x 5 mm, polished with granullometry N_o 400 and 600 SiC papers were used for immersion in the microbial cultures, after degreasing with acetone.

Table II. Chemical Composition of the Al Alloys (w/w%)

Tabla II. Composición química de las aleaciones de Aluminio (% m/m)

Alloy	Zn	Cu	Mn	Si	Mg	Fe	Cr	Ti	Al
7005	4.7	0.05	0.45	0.10	1.2	0.18	0.16	0.03	Rest
7075	5.2	1.6	0.09	0.23	2.6	0.24	0.13	0.02	Rest
2024	0.12	4.5	0.53	0.14	1.5	0.29	—	0.02	Rest
Pure Al	—	—	—	—	—	—	—	—	99.9

2.3. Methods

2.3.1. Drop test

SEM-EDX was used to evaluate the alloys' MIC increased susceptibility with the successive fungal generation cultures of the strain, initially non-evidencing corrosive behaviour. This susceptibility was followed through the drop test. It consists on putting in a Petri dish with sterile mB-H solution a metal sample polished up to 0.25 mm diamond paste. With all sterilised instruments and in a laminar flow cabinet, a piece of the metal sterilised in alcohol, dried by quick passage on a flame, was inoculated with a small piece of mycelium carefully cut with a tweezers. Placed the culture at the interphase of the mB-H nutrient solution and jet A1 fuel each Petri dish was covered and metal seeded with the microbe was maintained at 34 ºC [12, 13, 17 and 17] up to 10 days.

2.3.2. SEM – EDX analysis

After 10 day's incubation the metal samples were retired from the cultures and allowed to dry on filter paper in the culture oven. Then EDX on attacked secondary phases was performed. After samples sputtering SEM observation were performed and micrographs of the microbe/metal aspect registered.

3. RESULTS

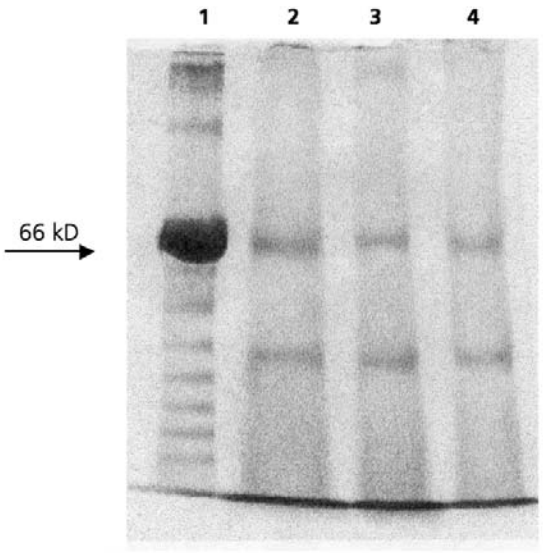
3.1. Biochemical analysis

A non-corrosive strain of the fungus *Hormoconis resinae* showed during first replication that neither the mycelia caused any corrosion on the tested pure Al nor on its three alloys. The result of protean profile of this first culture of non-corrosive strain grown in the presence of Al and the three Al alloys coincided with the control (culture grown in absence of the metals) in associated bands to the molecular weights 66 and 31 kDa, as can be seen in figures 1 and 2**.** An exception can be seen for AA 7075 alloy, which only presents one of these bands.

Referred to proteins present in the homogenate harvested from the different cultures of third generation, on the contrary, the results allowed to establish differences in the composition of the cellular extracts grown in the presence and absence of Al-base alloys as compared with the first generation culture (Fig. 3 and 4).

Then, the protein profiles of the newly grown mycelium showed differences in the protein composition depending on the different alloys presence or absence.

In tracks 1, 2 and 3, two bands may be observed coinciding in all three samples, one of which also appears in track 4. In track 5, two diffuse bands should also be similar to those found in 1, 2, 3 and 4 tracks. In track



Track 1: Molecular weight standard: BSA
Track 2: Control (1.21 mg ml^{-1} total protein)
Track 3: 2024 (2.22 mg ml^{-1} total protein)
Track 4: 7005 (1.84 mg ml^{-1} total protein)

Figure 1. Protein electrophoresis of the non-corrosive strain of the *Hormoconis resinae* mycelium, in presence and absence of AA 2024 and AA 7005, stained with Coomassie dye, first generation.

Figura 1. Electroforesis de proteínas de la cepa no corrosiva del micelio Hormoconis resinae*, en presencia y ausencia de AA 2024 y AA 7005, teñida con tinción de* Coomassie*, primera generación.*

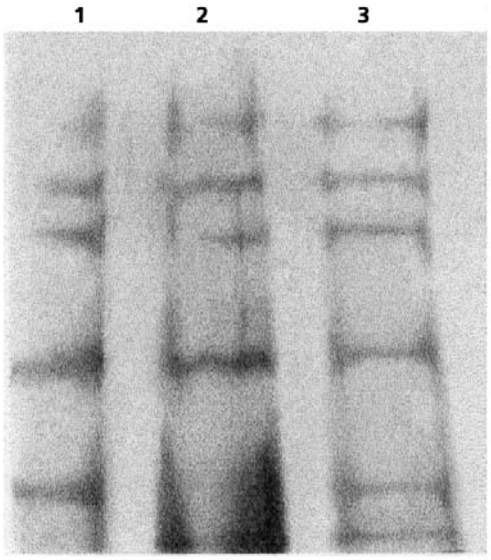


Figure 2. Protein electrophoresis of the non-corrosive strain of the *Hormoconis Resinae* mycelium, in presence and absence of pure Al and AA 7005, stained with silver dye, first generation**.**

Figura 2. Electroforesis de proteínas de la cepa no corrosiva del micelio de Hormoconis resinae*, en presencia y ausencia de Al puro y AA 7005, teñida con tinción plata, primera generación.*

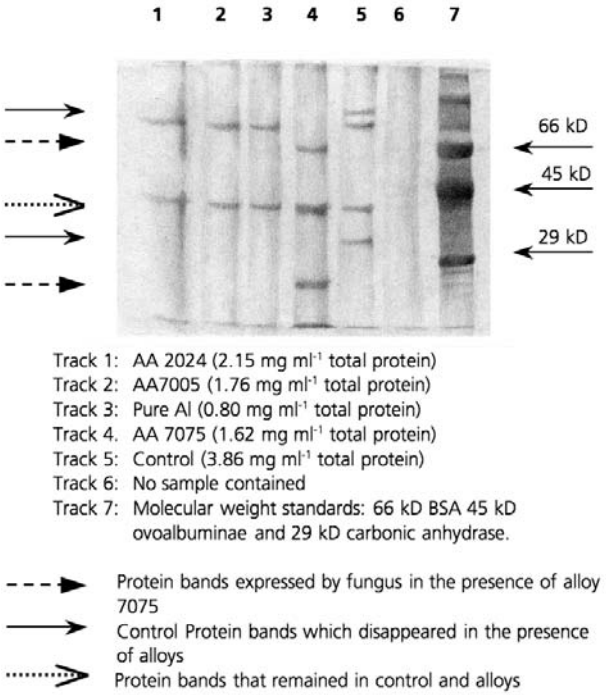


Figure 3. Protein electrophoresis of the *Hormoconis resinae* mycelium, in presence and absence of pure Al and AA 7005, stained with silver dye, third generation (corrosiveness recovered).

Figura 3. Electroforesis de proteínas del micelio de Hormoconis resinae*, en presencia y ausencia de aluminio puro y AA 7005, teñida con tinción plata, tercera generación (corrosividad recuperada).*

4, an obvious difference with respect to the other tracks is observed. In this case three well defined bands, the first one of which coincides with the band of the standard molecular weight corresponding to 66 kDa. The second band coincides with the second band observed in 1 to 3 track samples and the third band does not coincide with any other sample bands.

In figure 4 the protean bands expressed by *Hormoconis resinae* have been summarized for the first and third generations with their respective molecular weight. The results indicate that all the first generation present a band at 66 kDa, disappearing in the third generation samples, except when *Hormoconis resinae* is cultured in the presence of the AA 7075 alloy. Certainly, the cultures grown at the third generation produced a lower amount of protean bands. The tendency is that the molecular weight values of the small proteins in the first generation is higher in the third generation, except for the exhibited in the presence of AA 7075. Changes observed in the molecular weight bands of *Hormoconis resinae* cultured in the presence de AA 2024 y AA 7005 are similar amongst them, but higher to those of the first generation, diverging from this tendency when *Hormoconis resinae* is cultivated in the presence of AA 7075. At the third generation in front to the AA 7075 alloys it expresses new proteins of lower molecular weight, in the order of 25 kDa and it maintains two bands (65 y 42 kDa), while bands of high molecular weight (84 y 78 kDa) disappear respect to the first generation.

For the third generation, the fungus grown in the absence of the alloys (control) presents two different bands (85 y 34 kDa) from those expressed by the same generation of the strain growing in the presence of the alloys the arrows point at the above described differences, as can be seen in figure 3 once it recovered its corrosive behaviour.

3.2. SEM – EDX analysis

EDX on table III shows the chemical composition of the main attacked second phase of the alloys, which reach for AA7005 to the highest Zn content (14.07 %) and which is much higher than the that in the matrix, as shown in table II (4.7 %). In table III the EDX corresponding to second phases of the AA 7075 evidenced areas also enriched in Zn, Mg and Fe.

After carrying out replication cultures up to a third generation, the strain of the fungus recovered the corrosive behaviour, the attack becoming very intense on the

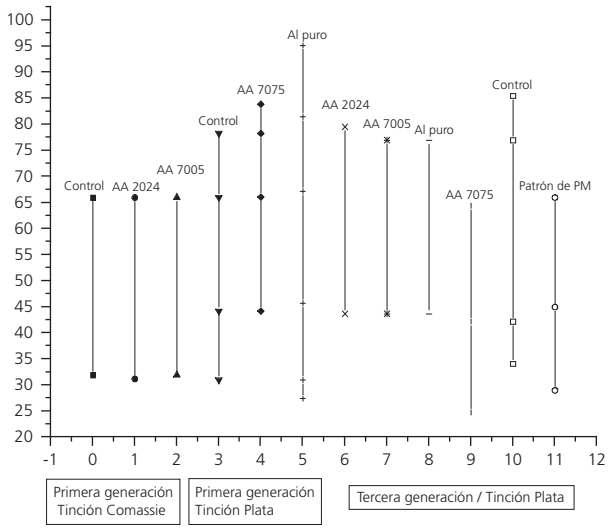


Figure 4. Molecular weight of proteins in the crude homogenate of the fungus *Hormoconis resinae* in the presence of aeronautical aluminium alloys.

Figura 4. Pesos moleculares de proteínas de los homogenados crudos del hongo Hormoconis resinae *en presencia de aleaciones aeronáuticas de aluminio.*

alloy secondary phases and following polygonal ways as in works for the previous corrosive behaviour [9, 11 and 17]. SEM aspect of the respective metal samples can be seen in figure 5 to allow comparison to the previous aspects under corrosive cultures[11 and 17].

Drop tests shows that the fungal mycelium produced only a soft attack on the alloys surface, pure Al was not affected and crystallographic attack nucleated preferentially on second phases.

4. DISCUSSION

SEM micrographs after the drop test on metal samples in figures 6a-6c reveal a very slight attack corresponding to non-corrosive behaviour produced by the mycelium was restricted to surface Al_2O_3 film damage and slight secondary phase dissolution below hyphae traces.

The displacement of hyphae is not polygonal, joining secondary phases, as it had been observed when corrosive behaviour occurred[17] Polygonal trace through second phases evidence a surface chemotactic or attraction effect on the mycelium, due to their higher local chemical content in certain elements throughout the matrix. This increased content in "oligoelements" respect to the matrix content would trigger the change in direction once fully up-taken the necessary element/s at a given second phase. Then the mycelium grows following a straight line up to the nearer second phase, up to 50 mm distances, to up-take again the same oligoelements[17].

Table III. EDX on second phases of the alloys W (%)

Tabla III. EDX de las segundas fases de las aleaciones W(%)

	AA 2024 EDX on a second phase	AA 7005 EDX on a second phase	AA 7075 EDX on matrix x 2500	AA 7075 EDX on a second phase x 5000
Al	60.40	85.94	88.66	78.73
Mn	2.48	—	—	—
Fe	9.83	—	—	1.24
Cu	27.30	—	3.04	4.92
Zn	—	14.07	8.31	11.33
Mg	—	—	—	3.77

In spite of their biochemical characteristics as oligoelements, they did not seem to exert any attraction effect on the displacement direction of the non-corrosive strain mycelia. Their attack is too slight to be the result of any up-take process by the first generation culture of this non-corrosive strain.

With the overfloating samples, a SDS-PAGE polyacrylamide electrophoresis was performed, as shown in figure 3. Therefore it can be inferred that the fungus grown separately in the presence of AA2024, AA7005 and pure Al presents the same band type, and the fungus grown in the presence of AA7075 presents two different bands from the other alloys, one of them coincides with the control sample of *Hormoconis resinae*. In the presence of this alloy the fungus of the 3rd generation maintains two bands (66 and 44 kDa) amongst those expressed by the fungus of the 1st generation. Moreover, it can be observed that when comparing the bands expressed by the fungus in 1st and third generation, two bands of the higher molecular weight (78 and y 84 kDa) dissapear and a new protein of lower molecular weight (25 kDa), is expressed. From this point of view and comparing to the proteins expression exhibited by the fungus of third generation in the presence of pure Al it is evident that the fungal mycelium grown in the presence of the AA 7075 alloy absolutely differs from the others samples in its protean expression. This fact could be explained analyzing the components of each alloy given that is that alloy which presents the highest mass % of those considered as "oligoelements", zinc, magnesium and iron. This fact could be interpreted as the absence of any physiological effect of either presence or absence of the metal phase when the fungal strain had lost its capacity to produce its original corrosive behaviour, as shown during the first generation. On the contrary, as it was previously demonstrated[15], when corrosiveness of a given strain has recovered at the third successive replication (or generation), the mycelium composition would express a different biochemi-

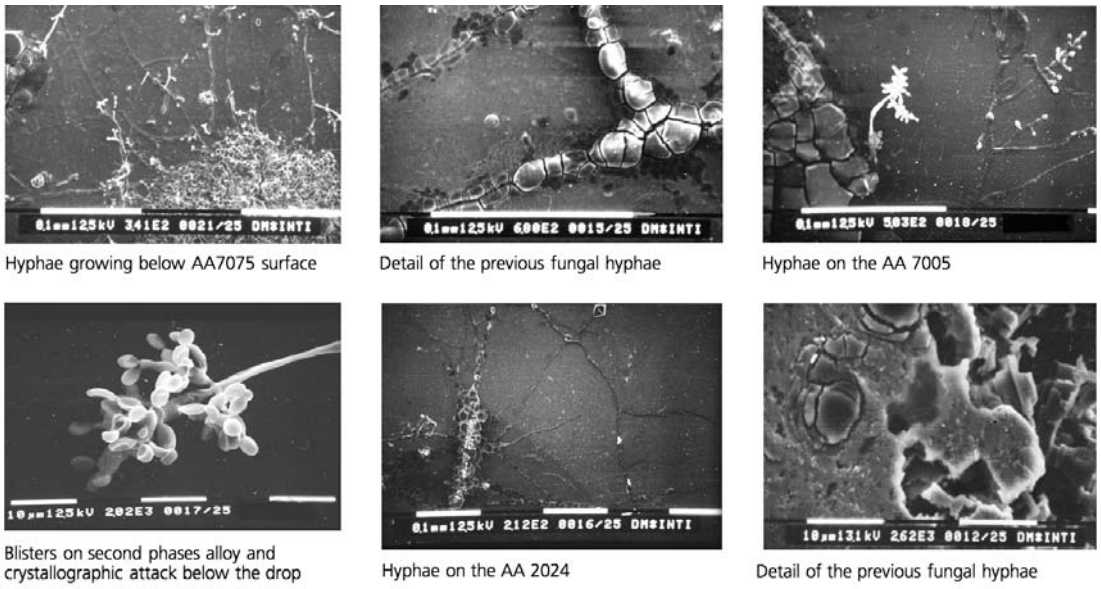


Figure 5. SEM aspect of the *Hormoconis resinae* corrosive strain attack on Al alloys[17].

Figura 5. Aspecto MEB del ataque de la cepa corrosiva de Hormoconis resinae *sobre aleaciones de Al [17] .*

cal response depending on the presence or absence of metallic Al and its three alloys.

This type of chemical differences could be considered an evidence of a biological driving force for microbial species ability of being or not corrosive to certain metal or alloy. The susceptibility to MIC of a given alloy would thus be modified on the basis of this biochemical knowledge for a corrosive microbial species present in a given service condition.

At present there is no information at international level to explain in biochemical terms fungi corrosiveness, nor the effect nutrients, included oligoelements, able to modify that characteristic.

However, from the results exposed in this paper it can be proposed that as well the lost as the recovered corrosiveness were correlative from reversible abilities of the fungus to adapt to different nutritional sources causing also simultaneous modification in the other referred characteristics. Both, the corrosiveness loss and recovery were associated to nutritional sources modification, from jet fuel as the only C source to malt agar extract and vice versa. The strain only seems to have modified its characteristics and behavior according the nutrients available.

By analogy to the advance attained from measurements of metallic-uptake by *the Hormoconis resinae* fungus[17 and 18] compared to results reported for different bacterium/metallic-ion systems[19], it was interesting to take a similar reference study, though with bacteria[26 and 27]. Zinkevich *et al*.[28] report that the presence or absence of metallic iron in the cultures of two different sulphate-reducing bacteria (SRB) strains induced exopolymer production of different chemical composition, which in this case it occurs in carbohydrate biomolecules. This culture regulation of the microbe physiological response to the metal presence at molecular level could be the answer also found for the fungus.

In the presence of toxic-ions as those of Hg, As, etc.[19] increased extracellular polymeric substances (EPS) production stimulated their selective up-take decreasing concentration in the medium, to allow survival of responsible bacterium during this "detoxification" processes. EPS production was reported as a physiological response provoked by high toxic ion concentrations, as Brown and Lester reported through the many examples exposed about "metal up-take" by bacteria in their review paper, already in 1979. Such information would be useful as a basis to demonstrate microbial species selection for bio-extraction of metal ions contaminating waters and soils.

On the contrary, it is likely that this ion up-take mechanism could differ from the one responsible for metallic corrosion. EPS secreted by corrosive species can contribute to the corrosive process not only by facilitating irreversible cell attachment leading to metal surface colonization but also by binding metal ion species, as demonstrated for SRB by Beech and Cheung in 1995 [29]. Also differences encountered in the EPSs chemical composition secreted by SRB into the bulk solution and within the biofilm[29] would support the fact that the EPS composition could be strongly affected by the proximity to the metal surface.

MIC seems to depend from the microbial capacity to produce EPSs able to colonise a given metal surface through atom up-take and removal from the lattice. This would also imply an oxidation step for ions irreversible attachment. In our case, the analogous role of bacterial

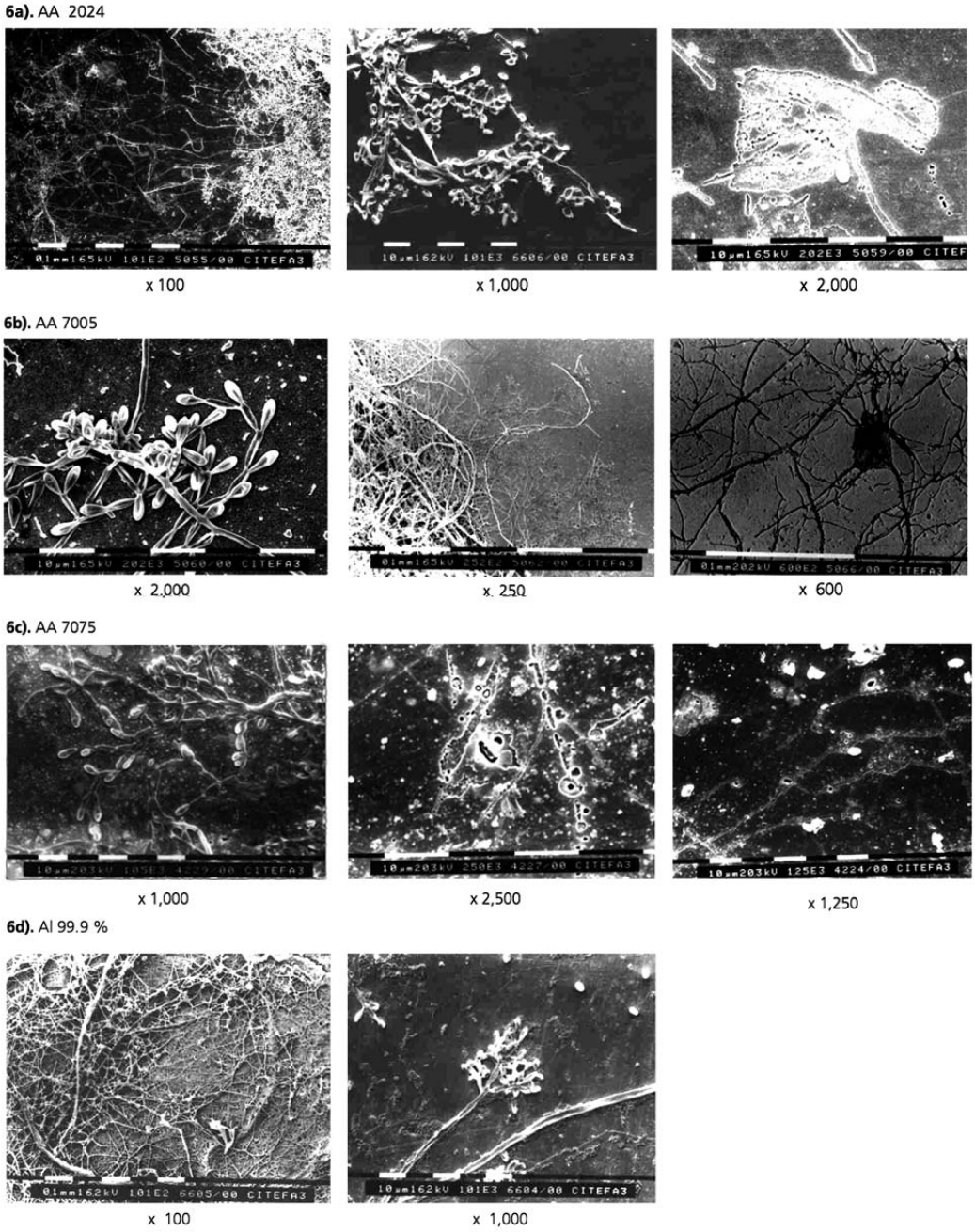


Figure 6. SEM aspect of the non-corrosive strain of *Hormoconis resinae* on: a) AA 2024 alloy. b) AA 7005 alloy. c) AA 7075 alloy. d) Pure aluminium.

Figura 6. Aspecto MEB de la cepa no corrosiva de Hormoconis resinae sobre: a) La aleación AA 2024. b) La aleación AA 7005. c) La aleación AA 7005. d) Aluminio puro.

EPS should be assigned to the fungal mycelium, whose local adherence and corrosiveness to the metal surfaces have been reported to determine the attack localisation and intensity in service military aircraft[12, 13 and 17].

The mentioned difference in the protean profile of the mycelia grown in the presence and absence of Al and its alloys should also control distinct type and rate of acid formation, not only through Jet A1 degradation but also by autolysis of mycelia of different age cultures, previously studied trough electrochemical and surface analysis techniques with very corrosive strains of *Hormoconis resinae*[6-9 and 13-18]. The main results revealed in those papers are a great effect of the fungal culture on: a) the pitting potential decrease of the Al-base alloys, b) the increasing current density of oxygen reduction and c) the pH decrease with incubation time of the modified B-H diluted nutrient medium.

Other manifestation of the existence of a biochemical response of the fungus to metal surface composition could be noticed comparing the hyphae aspect shown in

figures 6a-6c. SEM micrographs of the mycelium growing on the alloys and on pure Al respectively, above 1,000 magnifications, reveal that pure Al did not stimulate fungal sporulation while the alloys promote it.

These results provide new evidence about the corrosion risk of military aircraft in service due to the high surface area to culture volume of *Hormoconis resinae* ratio in heavily contaminated integral fuel tanks[12, 13 and 30].

5. CONCLUSIONS

A non-corrosive strain of the fungus *Hormoconis resinae* showed during its first replication-that the mycelia neither caused any corrosion on the pure Al nor on the AA 2024, AA 7075 and AA 7005 alloys.

In the present study only the protean biomolecules of the mycelia manifested variations associated to the corrosiveness as well as to the presence or absence of Al alloys metal samples in the cultures.

At the third generation, coinciding with the recovery of the lost corrosiveness, differences in the protein composition of the mycelia appeared when the fungal strain was grown in presence or absence of the alloys. This differences, non-detected for the first generation of the same non-corrosive strain, indicate that when the corrosive behaviour was recovered the fungal mycelium expressed some proteins in absence of alloys (control of the third generation), not present when the fungus grew in the presence of any alloy.

This evidence demonstrates a significant effect of the metal surfaces on enhancing the corrosive behaviour of the strain likely related to fungal metabolic needs.

This was also verified through morphological changes observed on the fungal hyphae depending on the metallic substratum colonised.

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