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Short Communication

Desoxirrobonuclease Activity in Clinical *Mycoplasma fermentans* Isolate

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Abstract

Mycoplasmas are a heterogeneous group of the smallest organisms capable of self replication, some mycoplasmas cause respiratory or urogenital diseases in humans. However, mycoplasmas often chronically colonize our respiratory and urogenital tracts without apparent clinical significance. In this respect, wall free mycoplasmas are among the few prokaryotes that can grow silently in close interaction with mammalian cells for a long period of time. However, prolonged interactions with mycoplasmas of seemingly low virulence could, through a gradual and progressive course, significantly affect many biologic properties of mammalian cells. DNases are important constituents of mycoplasmal membranes and may be involved in the acquisition of host nucleic acids required for growth. The objective was detected DNase activity in clinical Mycoplasma fermentans isolate. Digestion products were analyzed by loading a Tris-borate agarose gel, absorbance and medium for DNase test. DNase activity was observed in strains tested and the levels of activity varied, Mycoplasma fermentans P140 showing greater activity. Results suggest DNase activities may be essential for growth and survival, this finding suggest that there is a mechanism of nucleotide acquisition.

Key words: DNase, nucleotide acquisition, clinical sample, *Mycoplasma fermentans.*

Introduction

Mycoplasmas are a heterogeneous group of the smallest organisms capable of self-replication, some mycoplasmas cause respiratory or urogenital diseases in humans. However, mycoplasmas often chronically colonize our respiratory and urogenital tracts without apparent clinical significance. Wall-free mycoplasmas are among the few prokaryotes that can grow silently in close interaction with mammalian cells for a long period of time. However, prolonged interactions with mycoplasmas of seemingly low virulence could, through a gradual and progressive course, significantly affect may biologic properties of mammalian cells[1-4].

Mycoplasma fermentans commonly isolated from human genitourinary and respiratory tracts. It has been implicated in HIV pathogenesis, sexually transmitted genital tract infection, systemic infections, rheumatic disorders and chronic fatigue syndrome[5], notwithstanding the clinical significance, the roles it plays and the molecular mechanisms involved in various diseases have yet to be elucidated.

Nuclease activity in mycoplasmas has been proposed as the mechanism by which these organisms acquire the precursors required for their nucleic acids. Therefore, mechanisms must be present to induce release, degradation, and transport of host nucleic acids to intracellular sites. Transports processes have been identified in mycoplasmas[6,7], but DNA and RNA degradation processes have not received adequate attention. This study was undertaken to expand our knowledge about the desoxirribonuclease activity in clinical mycoplasmas isolate.

Material and Methods

Mycoplasma fermentans PG18 strain (ATCC 19989), and *Mycoplasma fermentans* P140 was isolated in our laboratory from the human respiratory tract. The organisms were grown in SP4 medium (mycoplasma broth base, tryptone, peptone, arginine, phenol red, DNA, supplemented with horse serum, CMRL 1066, yeast extract, yestolate, and glucose). The cultures were grown for 24-48 h at 37°C. Growth was monitored by measuring the OD 640 of the culture and by recording pH changes.

Mycoplasmas cultures were harvested at the mid exponential phase of growth by centrifugation at 12000 g for 20 min, washed once with 0.01 M sodium phosphate-0.14 M sodium chloride (pH 7.3) (PBS), and resuspended at a concentration of 100 µg of protein per ml in PBS containing 1% dextrose, 2 mM $CaCl_2$, and 2 mM $MgCl_2$ (PBS-CM). Protein concentration was determined with Bio-Rad dye reagent, by using bovine serum albumin as a reference standard. To determine activity, 25 µl of suspended cells, representing 2.5 µg of protein, was diluted in twofold increments in a 96-well plate with PBS-CM. An equal volume lamda DNA (10µg/ml of PBS) was added to each well, and the plate was then incubating for 24 h at 37°C. Lamda DNA

digestion was stopped by adding 7 µl of borate sample buffer ti each well. Digestion products were analyzed by loading a Tris-borate agarose gel with 9 µl of sample per well, running the gel at 100 volts for 45 min, and then staining it with ethidium bromide. The end point was determined by analyzing the reaction mixtures on agarose gels and identifying the time at which the undigested, full-length lamda DNA first disappeared.

Results and Discussion

In the present study, we examined DNase activity has been proposed as a mechanism for nucleic acid precursors acquire free bases and/or oligonucleotides, so the capacity of some mycoplasma species cells suggests that invade the host cell DNA serves as a substrate for the activity of nucleases and obtaining consistent precursors[8].

DNase activity assays show that *Mycoplasma fermentans* PG18 reference strain DNA degrades by less than *Mycoplasma fermentans* P140 clinical isolate. From 12 hours the *Mycoplasma fermentans* presents a more pronounced degradation gradient as they advance 24 hours as shown in figure-1, increased DNase activity by the isolate (P140) is explained by the recent interaction with host, a situation that affects more virulent in some clinical samples.

Figure-1

DNase activity products on agarose gel Tris-borate, lane 1 without digestion (control), lanes 2, 3, 4 and 5 with 6, 12, 18 and 24 hours respectively in presence of *Mycoplasma fermentans* PG18, lanes 6, 7, 8 and 9 with 6, 12, 18 and 24 hours respectively in presence of *Mycoplasmas fermentans* P140. It is noted that at 24 hours (lane 9) *Mycoplasma fermentans* P140 DNase activity degrades in 95% DNA, and *Mycoplasma fermentans* PG18 with 45% degradation (lane 5)

Absorbance readings confirm gradients observed in agarose gels of products from DNA degradation, DNA degradation due to the activity of DNase in *Mycoplasma fermentans* PG18 was 45% and *Mycoplasma fermentans* P140 at 24 hours was 95% (figure-2).

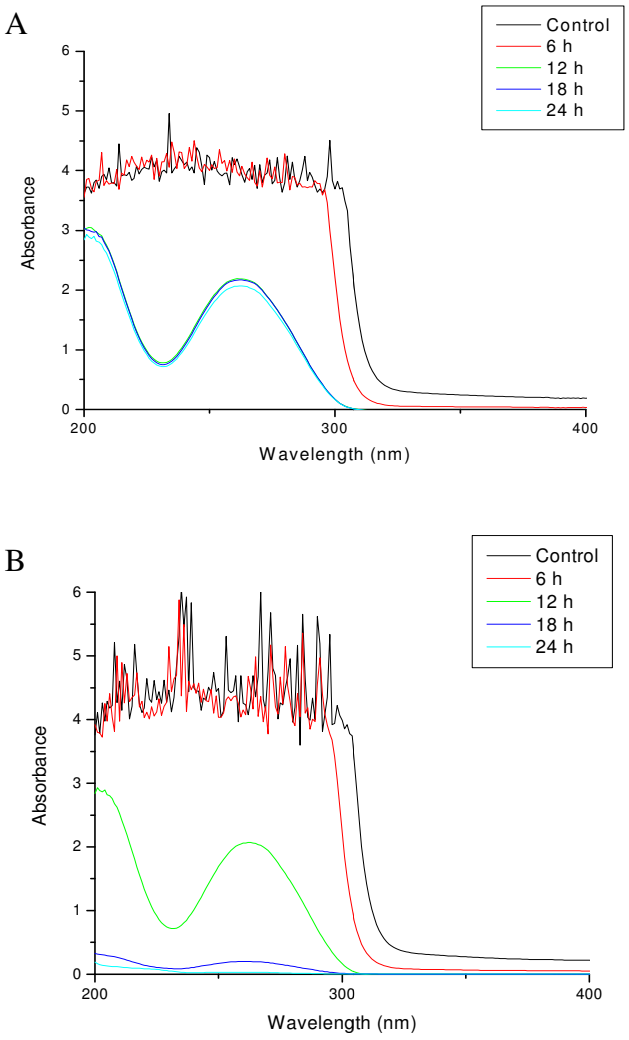


Figure-2

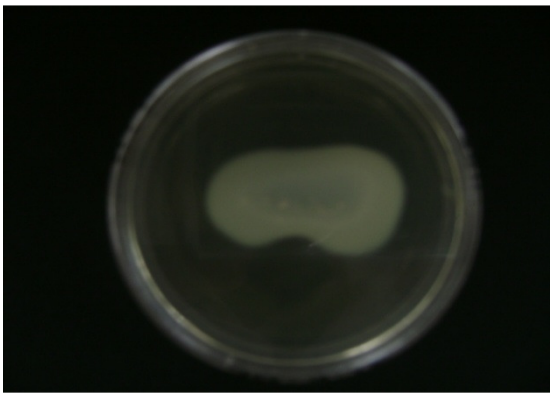
Absorbance values of DNA degradation products by DNase activity, (A) *Mycoplasma fermentans* PG18 with 45% degradation at 24 hours and (B) *Mycoplasma fermentans* P140 showed 95% DNA degradation to 24 hours

In agar culture assay showed a clear zone on microbial growth, indicative of enzymatic digestion, still more evident in the samples from the clinical isolate of *Mycoplasma fermentans* P140 (figure-3).

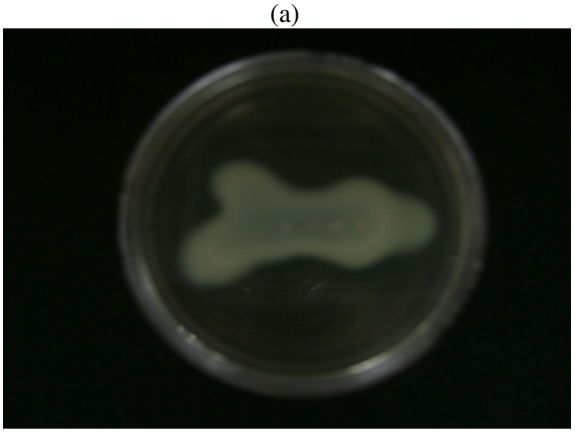
These data suggest that the DNase activity is essential in the growth and survival of *Mycoplasma fermentans*, principally in the clinical isolate, representing nucleotide acquisition mechanism, besides confirming that reported for *Mycoplasma pulmonis*, *Mycoplasma hyorhinis, Mycoplasma gallisepticum* and *Acholeplasma laidlawii* that are not medical importance[9-12].

Isolation of *Mycoplasma fermentans* from different tissues and their involvement in the pathogenesis of human

immunodeficiency virus can give the clinical importance since their participation in different pathologies have not widely studied[13,14], these data being a contribution in understanding some of the mechanisms that allow *Mycoplasma fermentans* be a successful parasite, besides representing a relevant data to carry out the etiology and in the determination of virulence of the bacterial strain isolated in the laboratory.



(a)



(b)

Figure-3

DNase activity assay on agar showed a clear zone on microbial growth considered positive reaction, (A) *Mycoplasma fermentans* PG18 y (B) *Mycoplasma fermentans* P140

Conclusion

Data presented enable *Mycoplasma fermentans* to consider more as a microorganism with DNase activity, this helps us better understand and expand the understanding of the mechanisms by which this microorganism, and also to understand how survives in different human tissues which have been isolated and related etiologic agent. They are studying other species of medical interest to establish its clinical importance and suggested to be applied more often laboratory diagnosis.

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