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Reviving the Coccoid Forms of *H. pylori* Serotypes

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***Helicobacter pylori*, the most common chronic bacterial infection, has shown in two significant structure; bacilli and coccoid form. The coccid form has been detected as a viable but not culturable form which forms in unfavorable situation to survive. This feature leads some problems especially during several sub-culturing the examining H. pylori serotypes. We were targeting this problem, based on finding a suitable environment for conversion the coccoid form to viable and culturable bacilli form.**

Key words: *Helicobacter pylori*, coccoid form, revive, in vivo medium.

Helicobacter pylori is gram negative microorganism, microaerophilic that were colonized in stomach of approximately half of the world wide population, and case chronic gastritis, duodenal ulcers, MALT lymphoma and gastric cancer[1].Three different forms of *H. pylori* has been detected; a viable spiral form, a viable coccoid form and a viable but not culturable form[2-6] Although the coccoid form of *H. pylori* had been detected in both human stomach[7] and natural environments[8], it has been observed that *H. pylori* can transform from a cultivable spiral-shaped to an non-cultivable coccoid form in undesirable conditions. Aging, starvation, expose to air, prolonged incubation, improper temperature, proton pump inhibitors and antibiotic treatment are different unfavorable conditions which may lead to conversion spiral to coccoid form[9].

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So far, six *H. pylori* serotypes (O1 to O6) had been detected that serotype O2 LPS not express lewis antigen of blood group[10].

Coccoid form of *H. pylori* is recognized as viable but not-cultivable *H. pylori* and in this study we intent to revive coccoid forms of *H. pylori*[12]

METHOD AND MATERIALS

Bacterial strains and culture. *H. pylori* strains ATCC 700392, SS1(Sydney Strian - custer, Holland), O2 serotype (awarded by Dr Graham, University of Washington, Seattle, Washington, Baylor College of Medicine and Houston, Texas, USA) and clinically isolated strains were used in this study. All *H. pylori* strains were cultured on Brucella agar plates with selective complements and incubated at 37°C in a CO_2 incubator (memert, Germany), in a microaerophile situation, until the desired amount of growth was obtained after 3-5 days . The strains were identified as *H. pylori* based on the biochemical tests, microscopic morphology and PCR. A single primer pair was used to amplify *H. pylori* ureC gene based on Gene Bank to approve of *H. pylori* genus. F: 5′-CCCTCACGCCATCAGTCCCAAAAA-3′and

R: 5′-AAGAAGTCAAAAACGCCCCAAAAC-3′ that enable amplify fragment to length of 417 bp (Fig. 1)[12].

Two series of all strains were used in this study. The first group was stored in -70°C, in Brucella broth medium, containing supplements and 30% glycerol, for four years and the second group were incubates (3-5 hours) in air for studying the effects of aging and air on the conversion of the bacilli form of *H. pylori* to coccoid form, respectively.

In vivo culture. To examine in vivo re-conversion of coccoid *H. pylori* to bacilli and spiral form, bacterial suspension of coccoid *H. pylori*, obtained under the induction situation in the previous experiment, was fed to BALB/c mice three times with 1×10^8 CFU/ml *H. pylori* serotypes with coccoid forms, by gavage. Distinguishably, for neutralizing the acidic environment of stomach and surviving *H. pylori* strains in the mice stomach, 3% Sodium-bicarbonate was added to bacterial suspension. After ten-day, mouse groups, victim then the stomach, in order to prepare different dilutions (10^{-1} to 10^{-4}), were extracted and cultured on the brucella agar medium. The plates were incubated at 37°C micro-aerophilic incubator and quantitation of CFU was performed 10 days.

We confirm the strains by biochemical, morphological and molecular tests.

Table 1. The results of culture *H. pylori* serotypes with coccoid forms 10 days after inoculation in BALB/C model by oral

Serotpes	CFU/mlH. pylori Inoculation by oralDuring Three times	CFU/mlNumber of *H. pylori*, 10 days after inoculation
SS1	3×10^8	10^{11}
ATCC700392	3×10^8	10^8
Clinical	3×10^8	10^9
O2	3×10^8	10^8

CFU: Colony Formation Unit, ml: milliliter, NC: None Cultured

RESULTS

Determination of coccoid forms under various environmental conditions. Bacterial morphology was analyzed in Gram-stained by optical microscope, after facing different environmental situation. All *H. pylori* strains (ATCC 700392, SS1, O2 serotype and clinically isolated strains) converted from spiral or bacilli to coccoid form. None of strains were grown on the enriched brucella agar medium.

The total number of bacteria was determined on plates of different dilutions (between 10^{-1} to 10^{-4}), for each strain (Table 1).

Fig. 1. Culture

Fig. 2. Urease

CONCLUSION

Helicobacter pylori have been detected in different morphological forms. In human stomach, it lives almost in bacilli form as a dominant form. Cocooid form of *H. pylori* is an adaptive form that by exposing to unfavorable situation such as aging, high temperature, cold starving, aerobiosis, prolonged incubation and others, may convert to this form.

So far investigations in field of reduction of coccoid forms have not reported but for optimization of *H. pylori* culture were published paper several ([12-19]).

In study, optimization of Helicobacter pylori culture was carried by Mohabati Mobarez et al in Tarbiat Modares University, Tehran, IRAN, in order to prepare favorable antigens set up and modified medium for both coccoid and spiral form of *H. pylori* which were observed in stomach while coccoid forms viable but non culturable[20]. Conversion to coccoid form is a escape tactic from undesirable conditions which microorganisms exert to survive[21, 22].

According to the conversion of the spiral and bacilli form of *H. pylori* to coccoid state, by exposing to unsuitable situation, we exerted different ways for reviving and reconverting the organism to culturable form. In previous study we were able to inhibit the formation of coccoid form by optimizing *H. pylori* growth medium and subsequently in recent study we revived coccoid forms in BALB/c stomach(Table 1).Revived rate in H.pylori ss1 equival to 0.003log,ATCC700392 equival to 3log,Clinical equival to 3.3log, Serotype O2 equival to 3log.

H. pylori ss1 because of mouse adaptation revived rate is more than others.

In this study we used mouse gastric environment as in vivo culture medium. Significantly, Helicobacter pylori serotypes were able to convert to revived and culturable form, in this natural condition. During a period of ten days, *H. pylori* serotypes adapt to in vivo condition which can probably repair the enzymatic deficiency. Collectively, we recommend using in vivo medium to revive the studying *H. pylori* serotypes. Subsequent to antigen variation, coccoid form face alteration in LPS and proteins profile, indeed reviving serotype supposed to be necessary for scientists.

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