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## ***Candida* accommodates non-culturable *Helicobacter pylori* in its vacuole - Koch's postulates aren't applicable**

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### Abstract

The following are the responses to the "letter to the editor" ("*Helicobacter* is preserved in yeast vacuoles! Does Koch's postulates confirm it?") authored by Nader Alipour and Nasrin Gaeini that rejected the methods, results, discussions and conclusions summarized in the review article authored by Siavoshi F and Saniee P. In the article, 7 papers, published between 1998 and 2013, were reviewed. The 7 papers had been reviewed and judged very carefully by the assigned expertise of the journals involved, including the reviewers of the *World Journal of Gastroenterology* (WJG), before publication. In the review article, 121 references were used to verify the methods, results and discussions of these 7 papers. The review article was edited by the trustworthy British editor of the (WJG), and the final version was rechecked and finally accepted by the reviewers of (WJG). None of the reviewers made comments like those in this "letter to the editor", especially the humorous comments, which seem unprofessional and nonscientific. Above all, the authors' comments show a lack of understanding of basic and advanced microbiology, e.g. bacterial endosymbiosis in eukaryotic cells. Accordingly, their comments all through the letter contain misconceptions. The comments are mostly based on personal conclusions, without any scientific support. It would have been beneficial if the letter had been reviewed by the reviewers of the article by Siavoshi and Saniee.

**Key words:** *Helicobacter pylori*; Intracellular occurrence; *Candida* yeast; 16S rDNA detection

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**Core tip:** The authors of the "letter to the editor" "*Helicobacter* is preserved in yeast vacuoles! Does Koch's postulates confirm it?" argue that amoeba fits better than *Candida* yeast in our review. They like to

see intracellular *Helicobacter pylori* only inside amoeba, not in yeast. The questions raised are: which one can carry *H. pylori* to the human gastrointestinal tract, vagina and skin, while remaining alive and able to colonize - amoeba or yeast? Which one is recognized as a member of the microbiota of these locations - amoeba or yeast? Accordingly, the authors present their personal conclusions, without supporting references and experimental data.

Siavoshi F, Saniee P. *Candida* accommodates non-culturable *Helicobacter pylori* in its vacuole - Koch's postulates aren't applicable. *World J Gastroenterol* 2018; 24(2): 310-314 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v24/i2/310.htm> DOI: <http://dx.doi.org/10.3748/wjg.v24.i2.310>

## TO THE EDITOR

### Response to all the comments in the "letter to the editor"<sup>[1]</sup>

**Paragraph 1:** Line 2: the title of the review article is written incorrectly: "Vacuoles of *Candida* yeast behave as a specialized niche for *Helicobacter pylori*" is wrong. Lines 4-10: the whole impression is incorrect. The review paper described detection of *H. pylori* inside the vacuole of *Candida* yeast, but not penetration<sup>[2]</sup>. This was demonstrated by microscopic observations of bacterium-like bodies (BLBs) inside the yeast vacuole and detection of *H. pylori*-specific genes in the whole DNA extracted from the yeast. It was proposed that the non-culturable intracellular *H. pylori*, like other non-culturable intracellular bacteria, e.g., those in arbuscular mycorrhizal fungi<sup>[3]</sup>, could multiply inside the vacuole of yeast cells and be transmitted to the next generation<sup>[2]</sup>. Lines 11-15: the article was a review article and therefore did not contain methods. It is not clear what methodologies the authors of the letter did not agree with. It would be better to write the same letter of criticism to those journals that published the original papers. The presence of several BLBs inside the yeast vacuole was not accepted by the authors. We have never emphasized the number of bacteria in the vacuoles. Counting the number of BLBs is not always possible and was not the aim of our studies. The letter authors presented their personal conclusions, which were not supported by published references.

**Paragraph 2:** One of the best-known genera of yeasts is *Candida*. Furthermore, *Candida* yeast, *Candida* species and *Candida albicans* have been used throughout the review article and the original papers. *Candida* species, especially the *C. albicans* that we studied, were isolates from the oral cavity, stomach, vagina and foods, as mentioned in all the original papers<sup>[4-10]</sup>. There are no simple biochemical tests

for the identification of yeasts, like those for bacteria. Macroscopic and microscopic characteristics, growth on CHROMagar, PCR and PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) are the methods currently used. We used colony characteristics, microscopic morphology, formation of blastoconidia and growth on CHROMagar<sup>[11]</sup> for identification of *Candida* species, as mentioned in our original papers. According to reports, *Candida* species are the inhabitants of human skin and mucosal surfaces<sup>[12-14]</sup>, as also mentioned in our papers. Accordingly, we knew we were studying *Candida* yeasts, i.e., yeasts belonging to the genus *Candida*. *Candida* yeast is mentioned in the title of the review article, and *C. albicans* and *Candida* species are mentioned throughout the published works.

**Paragraph 3:** Our studies had nothing to do with Koch's postulates. The aim of our studies was to show that the yeast cell can serve as a specialized niche and environmental reservoir for *H. pylori*. We have used standard and routine methods, as mentioned in our published papers, which have been checked and accepted by the experienced reviewers of the original papers and the review article. The authors of the letter have answered their own question about Koch's postulates (if they were applicable): because *H. pylori* was not culturable, Koch's postulates were not applicable. The authors are asked to present references that have applied Koch's postulates for *H. pylori* or any other bacteria and amoeba. The authors have presented their personal conclusions, without any support from published references.

**Paragraph 4:** All the immunologic methods for production of IgY-Hp and purification of antibodies were performed according to standard protocols, mentioned with the relevant reference in the original paper<sup>[15]</sup>. IgY-Hp perfectly and specifically interacted with *H. pylori* antigens, as described in the reference papers we used<sup>[16-18]</sup>. Even detection of *Campylobacter* proteins/antigens in the yeast protein pool would be another valuable finding. Both Western blot and immunofluorescent microscopy showed the immunospecificity of the IgY-Hp<sup>[8,9]</sup>. The authors have presented their personal conclusions, without supporting references, such as those describing immunologic detection of microbial antigens by human serum as a routine, commercialized, feasible and affordable method.

**Paragraph 5:** The discussion of bacterial tropism to yeast extract (which is a powder added to culture media) is totally unclear and has nothing to do with our work. The authors neglected to consider that "yeast extract", even yeast, if added to culture media, still goes through sterilization in the autoclave that kills all the microorganisms, including bacteria and yeast. Their

references numbered 2<sup>[19]</sup> and 3<sup>[20]</sup> cited in the letter are irrelevant. Reference number 2<sup>[19]</sup> is about adhesion of *H. pylori* to yeast cells, and not yeast extract (the powder). Reference number 3<sup>[20]</sup> is related to co-culture of *H. pylori* with free-living *Acanthamoeba castellanii*, which maintained the viability of *H. pylori* for up to 8 wk, again irrelevant. Their references numbered 4<sup>[5]</sup> and 5<sup>[21]</sup> in the letter do not correlate with the sentences given. The examination of body fluids to look for *H. pylori* is not clear and again irrelevant to yeast extract, yeast or *H. pylori*. Again, these are personal conclusions, without any scientific support.

**Paragraph 6:** Several papers on the intracellular occurrence of bacteria inside fungi, such as arbuscular mycorrhizal fungi, have been published. We used the details of these studies<sup>[22-25]</sup> as the main references in our original papers. The fungal cell wall as a barrier to bacterial entry to the fungal cell has been discussed in detail by Gehrig *et al.*<sup>[26]</sup>. However, in our studies we did not discuss the entry of *H. pylori* into the yeast cell. We only reported the intracellular occurrence of *H. pylori* inside the yeast; bacterial entry was not the subject of our studies. The David Copperfield story mentioned by the authors is an exciting fantasy and a reminder of childhood. However, it is adulthood, knowledge, experience and hard work that give one the courage to discover the unknown fantasies in the microbial world, like the discovery that bacteria once entered primitive eukaryotic cells through phagotrophy, and ended up being digested, slaves or endosymbionts<sup>[27-29]</sup>, as discussed in our original and review papers. Apparently, the authors like to see intracellular *H. pylori* only inside amoebae, but not in yeasts. The questions raised are: which one can carry *H. pylori* to the human gastrointestinal tract, vagina and skin, while remaining alive and even being able to colonize - amoeba or *Candida* yeast? Which one is recognized as a member of the microbiota of the human gastrointestinal tract, vagina and skin - amoeba or *Candida* yeast? Furthermore, the authors compared the size of amoeba and yeast and recognized amoeba as more suitable for the accommodation of several bacteria, without any supporting references.

**Paragraph 7:** The authors have answered their own question. Intracellular bacteria become intracellular to avoid stresses, such as the host immune response and antibiotics<sup>[30]</sup>. *H. pylori*, whether inside yeast, amoeba or epithelial cells, becomes more resistant to antibiotics. It is not known whether internalized antibiotics that reach the vacuole of eukaryotic cells, yeast or amoeba, remain intact and effective. Comparing the effects of antibiotics on *H. pylori* cells inside yeast with those in amoeba was not the concern of our studies. These are the authors' personal conclusions, with no supporting references. The location of *H. pylori* on the yeast cell is not clear. Our concern was the intracellular existence of

*H. pylori* inside the yeast.

**Paragraph 8:** The authors' comments are not clear. The relationship between yeast-positive individuals and a higher frequency of *H. pylori* infection was not discussed in our papers. We studied the intracellular occurrence of *H. pylori* inside the vaginal yeast of expectant mothers, and proposed that transmission of vaginal yeast to newborns might increase the likelihood of oral and gastric colonization by yeasts acting as a reservoir of *H. pylori*<sup>[7]</sup>. We did not study the frequency of vaginal yeasts in normal and healthy women, which has been accurately reported by the expertise of the field<sup>[31]</sup>. The subjects of their references 5, 6 and 7<sup>[21,32,33]</sup> were not the concern of our studies, because these papers reported the frequency of yeast in special patients and not in those with *H. pylori* infection.

**Paragraph 9:** The contents of paragraph 9 are unclear and confusing, not being correlated with even the titles of their references 8-19 used in the "letter to the editor". In this paragraph, the authors hypothesized the transmission of *H. pylori* through water consumption and related to amoebae, which has not been documented yet. The personal conclusion of the authors was again to replace yeast with amoeba in the review paper of Siavoshi and Saniee, which was not their own research project. The reason for using at least 6 similar papers out of a total of 20 references to describe the occurrence of amoeba in water is not clear. It was again the personal conclusions of the authors, who insisted on proposing amoeba and not yeast as the reservoir of *H. pylori*, without any supporting references or personal experimental data. Their reference number 18 is a paper with plagiarized content by the letter authors that has been retracted from the journal *Helicobacter* through the appropriate authorities, which has been fully acknowledged by Nader Alipour himself.

**Paragraph 10:** The comment about anti-*H. pylori* therapy, including antifungal drug usage, is confusing. Extensive clinical trials might reveal whether antifungal therapy is effective against *H. pylori* or not. Their reference number 20<sup>[34]</sup> is not relevant to the subject of the review. Their reference 17<sup>[35]</sup> reports *Candida*-associated ulcer in only one patient without implication of *H. pylori* infection, so statistical analysis was not applicable. Personal conclusions have been presented, without being confirmed by supporting references.

**Paragraph 11:** All the arguments of the authors that have rejected the idea of yeast as the reservoir of *H. pylori* are based on their personal conclusions, without presentation of any supportive references. Copying the whole text of the review paper and only replacing the name yeast with amoeba, and also the authors' names (Siavoshi F and Saniee P) with their own names, shows that they agree with the methods that they

have rejected all through the "letter to the editor".

**Paragraphs 12 and 13 (conclusions):** We tried to respond to all the comments in the "letter to the editor", paragraph by paragraph. When we reached the last two paragraphs, we realized that all the arguments of the authors were based upon a paper containing plagiarized content that they had authored, and they were trying to justify their misconduct by persistently arguing that yeast must be replaced with amoeba in a research article written by other people (Siavoshi F and Saniee P). In their paper based on plagiarism, they only replaced yeast with amoeba, and changed the authors' names from Siavoshi F and Saniee P to Alipour N, Gaeini N, Taner A, Yildiz F, Masseret S and Malfertheiner P. The authors other than Alipour were not aware of the plagiarism. All other sentences in the text, photographs and references had been left unchanged. The journal *Helicobacter* retracted the article with the plagiarism through the appropriate authorities, which has been fully acknowledged by Nader Alipour himself.

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