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Editorial

Update on Vaginal Lactobacilli and Biofilm Formation

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Albert S Döderlein (a German physician) described in 1892 an organism that was isolated from a vaginal specimen of asymptomatic pregnant women. He named it Doderlein's bacillus and was later renamed Lactobacillus [1]. Döderlein further considered and decided to divide the bacterial groups that he founded into normal (Grade A: dominated by the vaginal Döderlein's bacillus) and abnormal (Grade C: dominated by other micro-organisms). He also argued that in the normal vaginal secretions, the combination of Döderlein's bacilli and acidity were essential to keeping the vagina free of pathogenic bacteria [1]. This concept was fundamental and still continues to be utilized by contemporary vaginal investigators.

Over the past 4 or 5 years the advent of competing technologies, high-throughput sequencing (Pyrosequencing), software for computational analysis and genus-specific quantitative PCR (qPCR) assays have validated significant observations made by Döderlein and contemporaries(the protective role of lactobacilli and the importance of endogenous and exogenous microbes in the pathogenesis of diseases) [2-5]. It has also helped us to identify and characterize the structure of each bacteria and to compare it to the micro-flora of other individuals. Today it is well established that the majority of vaginas from women of child bearing age are colonized by mainly 4 groups of lactobacilli: *L. crispatus, L. iners, L. jensenii, and L. gasseri* (this is not true across all racial, locations and ethnic groups) [6,7]. Additionally we know that the healthier vaginas harbors mostly *L. crispatus and L. jensenii*as recently reported by Ravel et al based on pyrosequencing analysis [8].

Human vaginal lactobacilli express vaginotropism and are mainly derived from the gut micro-flora that colonizes the vaginal nutrient-rich environment maintaining a stable vaginal flora [9]. The estrogen dependent stratified vaginal epithelium, rich in glycogen, is controlled by the physiologic ovarian activity. The endogenous degradation of glycogen makes glucose accessible. Vaginal lactobacilli are considered to be obligate homo-fermenters of glucose having as end product: lactic acid. Lactobacilli also produce hydrogen peroxide and with lactic acid they are responsible to maintaining vaginal pH between 3.8 and 4.2. Moreover lactobacilli also produce hydroxyl radicals and bacteriocins (small peptides with microbicide activity) which help in keeping other bacteria under control [10,11]. Other recognized lactobacilli secretions include organic acids, bio-surfactants, and arginine deaminizes that allowed specific receptors to adhere to the vaginal epithelium and co-aggregate to form biofilms [12].

Almost all lactobacilli have a small genome of approximately 1.8–2 Mb, due to a number of evolutionary adaptations including losses of genes for biochemical activities as well as acquirements of essential genes by horizontal transfer through bacterial conjugation and phage infection.[12]Such new genes have contributed to "protein transporter systems" that facilitates lactobacilli vaginal colonization. Mendes-Soares et al. found that the genomes of lactobacilli species that exhibit vaginotropism were significantly smaller and had significantly lower GC content than those of the nonvaginal species [13]. The genes related to the bacteriocin production were recently identified and sequenced by Stoyancheva et al. [14].

Biofilm Formation by Lactobacilli

Bacteria frequently live as complex conglomerates known as biofilms. In general, single-species biofilm formation involves two main independent steps: initial adhesion to the surface and biofilm accumulation [15].

Biofilm formation is a sophisticated process that includes the recognition of surface-related stimuli to enable adhesion and a matrix production that is extraordinarily complex in its structure and function. This matrix is identified as the extracellular polymeric substances (EPS) and within it bacteria are intertwined in.

Recently we have learned about the contribution of genetic determinants in biofilms formation and how the environmental conditions influence the process. Additionally it is now known that bacteria exhibit biofilm-linked traits and heightened tolerance to antibiotic treatment and host defenses [16].

Lately lactobacilli were studied by Gomaa for biofilm formation in different growth media. All lactobacilli isolate produced biofilm on polystyrene surface. *L. acidophilus* showed the highest biofilm formation. He reported auto and co-aggregation with three pathogenic bacterial strains [17].

Last year, Ventolini verbally presented at COGI in Vienna findings regarding biofilms produced by human vaginal lactobacilli (manuscript under consideration by Medical Hypothesis) [18]. Follow-up qPCR research categorized these lactobacilli as *L. jensenii* (to be presented at COGI in Paris). A microscopic photograph (normal saline vaginal wet mount, 400 x magnifications) is included as an early release Figure 1.

Conclusion

Lactobacilli are responsible for maintaining the natural healthy micro-flora balance in the vagina. Our knowledge has been progressive and we have now a more complete interpretation of the intricacies and interactions between the diverse vaginal micro-flora and lactobacilli. Clinical research is still necessary to identify the contributions that lactobacilli biofilm could make regarding preterm labor prevention and protection against recurrent bacterial and fungal infections.

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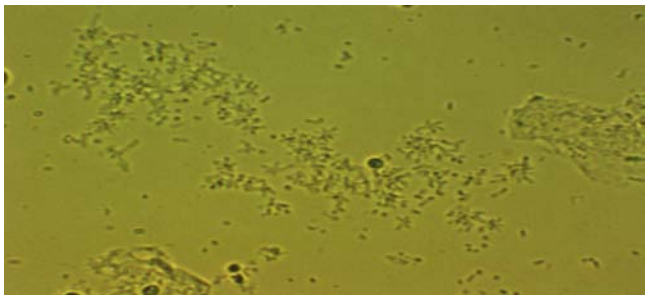

Figure 1: Biofilm production by Lactobacilli Jensenii in human vagina (normal saline mount).

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