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IN VITRO CULTIVATION OF *TREPONEMA PALLIDUM*: NEW TOOL TO STUDY PHYSIOLOGY AND GENETICS OF THE SYPHILIS AGENT

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Treponema pallidum subsp. *pallidum* is the causative agent of syphilis, a sexually transmitted human disease with over 6 million new syphilis cases per year worldwide[1]. There are two genetically distinct groups of strains denoted as Nichols-like strains and SS14-like strains[2,3].

T. pallidum is an obligatory human pathogen, which was considered to be uncultivable for decades[4]. Handful of treponemal strains has been isolated and maintained *in vivo* using passages in rabbits[5]. In 2018, Edmondson and colleagues published a revolutionary technique allowing *in vitro* cultivation of *T. pallidum* in laboratory conditions[6]. *T. pallidum* multiplies in a presence of rabbit epithellial cells and modified complete medium at 34 °C in microaerobic atmosphere (5% CO_2 and 1.5% O_2). Long-term cultivation requires regular subcultures every 7 days[7].

In our study, seven different *T. pallidum* strains (out of 16 known) from Nichols-like as well as from SS14-like group (n=3 and n=4, respectively) have been successfully adapted for *in vitro* cultivation system. Now, these strains are continually cultivated for more than two years.

Results obtained from *in vitro* cultivation revealed that average generation time ranges between 42.9 and 67.4 hours and significantly differs among various *T. pallidum* strains. Strain DAL-1 (from Nichols-like group) showed the fastest multiplication compared to all six other strains. Based on the growth patrameters and genetic differences of different strains, *in vitro* genetic manipulation was performed[8] and two genes/genetic regions involved in faster multiplication of DAL-1 strain has been identified.

Besides growth rates, set of *T. pallidum* strains has been characterized with respect to *in vitro* susceptibility to two clinically relevant antibiotics – penicillin and ceftriaxone. The average minimal inhibition concentration (>90% of growth inhibition) has been established as 0.0005 μg/mL and 0.005 μg/mL for penicilin and ceftriaxone, respectively.

Using *in vitro* cultivation, proteome for six different *T. pallidum* strains has been obtained and analyzed. Proteomic analysis determined more than 80% of predicted proteins from previous genomic studies. In addition, several abundant flagellar and outer membrane proteins were found among all six strains. These proteins represent candidates for syphilis vaccine design.

Taken together, we identified several physiological characteristics of *T. pallidum* using *in vitro* cultivation. This technique represents an important tool for better understanding of evolution and infection strategies of treponemal pathogens.

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