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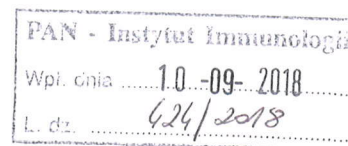
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Opinion

on doctoral dissertation of Adnan Saeed, MSc. entitled

**“Structural and immunochemical studies of polysaccharides from some
pathogenic actinomycetal strains”**

Presented dissertation of Mr Adnan Saeed has been performed in Laboratory of Medical Microbiology at the Institute of Immunology and Experimental Therapy of Polish Academy of Sciences in Wrocław under supervision of Professor Andrzej Gamian. According to the title, the doctoral thesis is devoted to structural and immunological studies of selected Gram positive pathologic bacterial strains which belong to genera: *Actinomyces* (*Actinomyces israelii*, *A. naeslundii*, *A. Odontolyticus*) and *Tsukamurella* (*Tsukamurella pulmonis*). These problems are very important subjects of scientific studies not only because of the possibility to obtain the data for better understanding of molecular mechanisms of infection and pathogenesis of the relevant diseases but also because their practical aspects i.e. essential usefulness in looking for better, more accurate diagnostic and therapeutic methods.

Mr Adnan Saeed's dissertation has been written in English according to scheme typical for experimental research projects in biological sciences. I do not feel to be competent to verify correctness of the language. Nevertheless, I can say that Author has used clear, understandable language and proper terminology. The entire work contains altogether 115 pages, 36 figures and photos, 10 tables and 119 literature sources cited. It is organized in 10 sections divided into detailed subsections. Exhaustive list of the used abbreviations is enclosed. The dissertation is composed of 10 main sections and contains lists of content, figures used abbreviations and abstract.

Introduction (section 1.) is a review type chapter describing bacterial genera *Actinomyces* and *Tsukamurella* particularly focusing on the up-to-date knowledge about chemical structure and functions of the bacterial cell walls. This chapter is a well written adequate text introducing to the matter and problems which are essential in the research and discussed in further parts of the work. It should be emphasised that Author cares about unspecialized readers giving clear, precise descriptions and explanations of the used abbreviations and listed them separately as well. Text contains adequate citations of the literature sources. Additionally, this section is illustrated by several figures well correlated with the text.

Aims of the research project are presented very clearly, however a bit too briefly, in section 2. As Author says the aim of this work was characterization of surface properties of the studied strains with particular attention to the immunochemical characterization of extracellular polysaccharides and their possible role in infection. At this point I would suggest giving a broader explanation of chosen methods for obtaining the expected results.

Used materials, chemicals, methods, obtained results and discussion of the results were given in the next separated sections.

In my opinion, the author's choice of the used methods and set of numerous performed experiments are very adequate to the project's aims. Number of the experiments performed is impressive indeed. The experiments allows to realize effectively the aims and to obtain new interesting data on the studied bacteria strains. Bacteria were identified by MALDI-TOF mass spectrometry and characterised by scanning electron-ion microscopy, adhesive techniques, measurements of hydrophobic properties of the surface etc. High usefulness of MALDI-TOF method to the identification of the bacteria were confirmed. The studied bacteria strains were cultured to obtained a source material for isolation and purification of some polysaccharides.

They were next investigated by many sophisticated physicochemical methods like NMR spectroscopy, various chromatography techniques, chemical and immunochemical analysis.

After description of the obtained results they are discussed in terms of their originality as well as their scientific and practical importance. In a separate section Author presents six conclusions of microbiological and clinical value of the results and also of usefulness of some used methods to such studies. I would prefer to see the conclusions organized and described in a hierarchical sequence from particular to more general ones.

In my opinion, providing of reliable, well documented data dealing with chemical and immunological properties of cell surface and isolated polysaccharides which allow to draw of the conclusions of medical importance is the most important achievement of the project. The obtained data are original in respect of the studied microbial species and markedly widen our knowledge in biology and immunology .

Surely, the investigation performed in this project and the obtained original results satisfy requirements for doctoral projects in excess.

After studying of the dissertation I am convinced that during his graduate and undergraduate studies Mr Saeed reached very good interdisciplinary knowledge, ability to use of proper scientific terminology in microbiology, skill in performing an effective research and ability to answer scientific questions.

Editing the text Author did not avoid some errors and defects. They are for example:

- numbering of the figures in section 1 is not in increasing sequence,
- lack or very poor legends of the plots on figures presenting NMR spectra
- no explanation of “molar ratio” (what it deals with) in Table 7,
- Fig. 17 – poor legend for the chromatograms, described fraction I in the text is not marked on,
- page 72 – the description of MALDI-TOF plots in indicate Fig. 21, however the MALDI-TOF plots are in Fig. 22,
- list of references (section 9. References) is supposed to be in alphabetical order according names of the first authors but it does not keep the rule.

The criticism above deals to formal shape of the text and it does not detract scientific of its value.

Summary

In short, I state that Mr Adnan Saeed have performed very interesting research which is important in biology of bacteria species and medicine as well. Big amount of data dealing with structural, functional and immunological properties of the studied strains from genera *Actinomyces* and *Tsukamurella* were collected. Author showed specialized knowledge in microbiology, biochemistry as well as good skills in using of various laboratory methods and techniques. The assigned scientific tasks were achieved. The obtained results are of great value and contribute to effort of understanding of molecular mechanisms of infectious diseases. Adnan Saeed proved he is already well prepared for work as a scientist in microbiology and related disciplines. It should be emphasized that the presented here results and conclusions were partly published and presented during scientific conferences. The thesis of Mr Adnan Saeed meets requirements for doctoral procedures and thus I recommend his thesis for further stages of doctoral proceeding.

I declare hereby that this doctoral thesis of MSc Adnan Saeed entitled "Structural and immunochemical studies of polysaccharides from some pathogenic actinomycetal strains" is according to rules for doctoral theses (Ustawa z dnia 14 marca 2003 r. O stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki, i późn. zmianami), therefore I apply to Scientific Council of Hirsfeld Institute of Immunology and Experimental Therapy of Polish Academy of Sciences in Wrocław for permission for further steps of processing of doctoral degree.

Wrocław, 5.09. 2018

Jan Gutowicz

