ABOUT AJMR

The African Journal of Microbiology Research (AJMR) (ISSN 1996-0808) is published Weekly (one volume per year) by Academic Journals.

African Journal of Microbiology Research (AJMR) provides rapid publication (weekly) of articles in all areas of Microbiology such as: Environmental Microbiology, Clinical Microbiology, Immunology, Virology, Bacteriology, Phycology, Mycology and Parasitology, Protozoology, Microbial Ecology, Probiotics and Prebiotics, Molecular Microbiology, Biotechnology, Food Microbiology, Industrial Microbiology, Cell Physiology, Environmental Biotechnology, Genetics, Enzymology, Molecular and Cellular Biology, Plant Pathology, Entomology, Biomedical Sciences, Botany and Plant Sciences, Soil and Environmental Sciences, Zoology, Endocrinology, Toxicology. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles are peer-reviewed.

Contact Us

Editorial Office: ajmr@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://academicjournals.org/AJMR
Submit manuscript online http://ms.academicjournals.me/
Editors

Prof. Fukai Bao
Department of Microbiology and Immunology
Kunming Medical University
Kunming 650031, China

Dr. Jianfeng Wu
Dept. of Environmental Health Sciences,
School of Public Health,
University of Michigan,
USA

Dr. Ahmet Yilmaz Coban
OMU Medical School,
Department of Medical Microbiology,
Samsun,
Turkey

Dr. Seyed Davar Siadat
Pasteur Institute of Iran,
Pasteur Square, Pasteur Avenue,
Tehran,
Iran.

Dr. J. Stefan Rokem
The Hebrew University of Jerusalem
Department of Microbiology and Molecular Genetics,
P.O.B. 12272, IL-91120 Jerusalem,
Israel

Prof. Long-Liu Lin
National Chiayi University
300 Syuefu Road,
Chiayi,
Taiwan

Dr. Thaddeus Ezeji
Assistant Professor
Fermentation and Biotechnology Unit
Department of Animal Sciences
The Ohio State University
1680 Madison Avenue
USA.

Associate Editors

Dr. Mamadou Gueye
MIRCE/ Laboratoire commun de microbiologie
IRD-ISRA-UCAD, BP 1386,
DAKAR, Senegal.

Dr. Caroline Mary Knox
Department of Biochemistry, Microbiology and Biotechnology,
Rhodes University,
Grahamstown 6140
South Africa.

Dr. Hesham Elsayed Mostafa
Genetic Engineering and Biotechnology Research Institute (GEBRI)
Mubarak City for Scientific Research,
Research Area, New Borg El-Arab City,
Post Code 21934, Alexandria, Egypt.

Dr. Wael Abbas El-Naggar
Head of Microbiology Department,
Faculty of Pharmacy,
Mansoura University,
Mansoura 35516, Egypt.

Dr. Abdel Nasser A. El-Moghazy
Microbiology, Molecular Biology, Genetics Engineering and Biotechnology
Dept of Microbiology and Immunology
Faculty of Pharmacy
Al-Azhar University
Nasr City,
Cairo, Egypt

Dr. Barakat S.M. Mahmoud
Food Safety/Microbiology
Experimental Seafood Processing Laboratory
Costal Research and Extension Center
Mississippi State University
3411 Frederic Street
Pascagoula, MS 39567
USA

Prof. Mohamed Mahrous Amer
Poultry Disease (Viral Diseases of poultry)
Faculty of Veterinary Medicine,
Department of Poultry Diseases
Cairo University, Giza, Egypt
Dr. Kamel Belhamel  
Faculty of Technology,  
University of Bejaia  
Algeria

Dr. Sladjana Jevremovic  
Institute for Biological Research  
Sinisa Stankovic,  
Belgrade,  
Serbia

Dr. Tamer Edirne  
Dept. of Family Medicine, Univ. of Pamukkale  
Turkey

Dr. R. Balaji Raja M.Tech (Ph.D)  
Assistant Professor,  
Department of Biotechnology,  
School of Bioengineering,  
SRM University,  
Chennai.  
India

Dr. Minglei Wang  
University of Illinois at Urbana-Champaign, USA

Dr. Mohd Fuat ABD Razak  
Institute for Medical Research  
Malaysia

Dr. Davide Pacifico  
Istituto di Virologia Vegetale – CNR  
Italy

Prof. Dr. Akrum Hamdy  
Faculty of Agriculture, Minia University, Egypt  
Egypt

Dr. Ntobeko A. B. Ntusi  
Cardiac Clinic, Department of Medicine,  
University of Cape Town and  
Department of Cardiovascular Medicine,  
University of Oxford  
South Africa and  
United Kingdom

Prof. N. S. Alzoreky  
Food Science & Nutrition Department,  
College of Agricultural Sciences & Food,  
King Faisal University,  
Saudi Arabia

Dr. Chen Ding  
College of Material Science and Engineering,  
Hunan University,  
China

Dr. Svetlana Nikolić  
Faculty of Technology and Metallurgy,  
University of Belgrade,  
Serbia

Dr. Sivakumar Swaminathan  
Department of Agronomy,  
College of Agriculture and Life Sciences,  
Iowa State University,  
Ames, Iowa 50011  
USA

Dr. Alfredo J. Anceno  
School of Environment, Resources and Development (SERD),  
Asian Institute of Technology,  
Thailand

Dr. Iqbal Ahmad  
Aligarh Muslim University,  
Aligrah  
India

Dr. Josephine Nketsia-Tabiri  
Ghana Atomic Energy Commission  
Ghana

Dr. Juliane Elisa Welke  
UFRGS – Universidade Federal do Rio Grande do Sul  
Brazil

Dr. Mohammad Nazrul Islam  
NIMR; IPH-Bangalore & NIUM  
Bangladesh

Dr. Okonko, Iheanyi Omezuruike  
Department of Virology,  
Faculty of Basic Medical Sciences,  
College of Medicine,  
University of Ibadan,  
University College Hospital,  
Ibadan,  
Nigeria
Dr. Giuliana Noratto  
Texas A&M University  
USA

Dr. Phanikanth Venkata Turlapati  
Washington State University  
USA

Dr. Khaleel I. Z. Jawasreh  
National Centre for Agricultural Research and Extension, NCARE  
Jordan  

Dr. Babak Mostafazadeh, MD  
Shaheed Beheshty University of Medical Sciences  
Iran

Dr. S. Meena Kumari  
Department of Biosciences  
Faculty of Science  
University of Mauritius  
Mauritius

Dr. S. Anju  
Department of Biotechnology,  
SRM University, Chennai-603203  
India

Dr. Mustafa Maroufpor  
Iran

Prof. Dong Zhichun  
Professor, Department of Animal Sciences and Veterinary Medicine,  
Yunnan Agriculture University,  
China

Dr. Mehdi Azami  
Parasitology & Mycology Dept,  
Baghaeie Lab.,  
Shams Abadi St.  
Isfahan  
Iran

Dr. Anderson de Souza Sant’Ana  
University of São Paulo.  
Brazil.

Dr. Julianne Elisa Welke  
UFRGS – Universidade Federal do Rio Grande do Sul  
Brazil

Dr. Paul Shapshak  
USF Health,  
USA

Dr. Jorge Reinheimer  
Universidad Nacional del Litoral (Santa Fe)  
Argentina

Dr. Qin Liu  
East China University of Science and Technology,  
China

Dr. Xiao-Qing Hu  
State Key Lab of Food Science and Technology  
Jiangnan University  
P. R. China

Prof. Branislava Kocic  
Specialist of Microbiology and Parasitology  
University of Nis, School of Medicine Institute for Public Health Nis, Bul. Z. Djindjica 50, 18000 Nis  
Serbia

Dr. Rafel Socías  
CITA de Aragón,  
Spain

Prof. Kamal I. Mohamed  
State University of New York at Oswego  
USA

Dr. Adriano Cruz  
Faculty of Food Engineering-FEA  
University of Campinas (UNICAMP)  
Brazil

Dr. Mike Agenbag (Michael Hermanus Albertus)  
Manager Municipal Health Services,  
Joe Gqabi District Municipality  
South Africa

Dr. D. V. L. Sarada  
Department of Biotechnology,  
SRM University, Chennai-603203  
India.

Dr. Samuel K Ameyaw  
Civista Medical Center  
United States of America
Prof. Huaizhi Wang  
*Institute of Hepatopancreatobiliary Surgery of PLA Southwest Hospital, Third Military Medical University Chongqing400038 P. R. China*

Prof. Bakhiet AO  
*College of Veterinary Medicine, Sudan University of Science and Technology Sudan*

Dr. Saba F. Hussain  
*Community, Orthodontics and Pediatric Dentistry Department Faculty of Dentistry Universiti Teknologi MARA 40450 Shah Alam, Selangor Malaysia*

Prof. Dr. Zohair I.F.Rahemo  
*State Key Lab of Food Science and Technology Jiangnan University P. R. China*

Dr. Afework Kassu  
*University of Gondar Ethiopia*

Prof. Isidro A. T. Savillo  
*ISCOF Philippines*

Dr. How-Yee Lai  
*Taylor’s University College Malaysia*

Dr. Nidheesh Dadheech  
*MS. University of Baroda, Vadodara, Gujarat, India. India*

Dr. Omitoyin Siyanbola  
*Bowen University, Iwo, Nigeria*

Dr. Franco Mutinelli  
*Istituto Zooprofilattico Sperimentale delle Venezie Italy*

Dr. Chanpen Chanchao  
*Department of Biology, Faculty of Science, Chulalongkorn University Thailand*

Dr. Tsuyoshi Kasama  
*Division of Rheumatology, Showa University Japan*

Dr. Kuender D. Yang, MD.  
*Chang Gung Memorial Hospital Taiwan*

Dr. Liane Raluca Stan  
*University Politehnica of Bucharest, Department of Organic Chemistry “C.Nenitzescu” Romania*

Dr. Muhammed Osman  
*Senior Lecturer of Pathology & Consultant Immunopathologist Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Malaysia*

Dr. Mohammad Feizabadi  
*Tehran University of medical Sciences Iran*

Prof. Ahmed H Mitwalli  
*State Key Lab of Food Science and Technology Jiangnan University P. R. China*

Dr. Mazyar Yazdani  
*Department of Biology, University of Oslo, Blindern, Oslo, Norway*

Dr. Ms. Jemimah Gesare Onsare  
*Ministry of Higher, Education Science and Technology Kenya*
Dr. Babak Khalili Hadad
Department of Biological Sciences,
Roudehen Branch,
Islamic Azad University,
Roudehen
Iran

Dr. Ehsan Sari
Department of Plan Pathology,
Iranian Research Institute of Plant Protection,
Tehran,
Iran.

Dr. Snjezana Zidovec Lepej
University Hospital for Infectious Diseases
Zagreb,
Croatia

Dr. Dilshad Ahmad
King Saud University
Saudi Arabia

Dr. Adriano Gomes da Cruz
University of Campinas (UNICAMP)
Brazil

Dr. Hsin-Mei Ku
Agronomy Dept. NCHU 250 Kuo
Kuang Rd, Taichung,
Taiwan

Dr. Fereshteh Naderi
Physical chemist,
Islamic Azad University,
Shahre Ghods Branch
Iran

Dr. Adibe Maxwell Ogochukwu
Department of Clinical Pharmacy and Pharmacy Management,
University of Nigeria,
Nsukka.
Nigeria

Dr. William M. Shafer
Emory University School of Medicine
USA

Dr. Michelle Bull
CSIRO Food and Nutritional Sciences
Australia

Prof. Dr. Márcio Garcia Ribeiro (DVM, PhD)
School of Veterinary Medicine and Animal Science-UNESP,
Dept. Veterinary Hygiene and Public Health,
State of Sao Paulo
Brazil

Prof. Dr. Sheila Nathan
National University of Malaysia (UKM)
Malaysia

Prof. Ebiamadon Andi Brisibe
University of Calabar,
Calabar,
Nigeria

Dr. Julie Wang
Burnet Institute
Australia

Dr. Jean-Marc Chobert
INRA- BIA, FIPL
France

Dr. Zhilong Yang, PhD
Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases,
National Institutes of Health

Dr. Dele Raheem
University of Helsinki
Finland

Dr. Li Sun
PLA Centre for the treatment of infectious diseases,
Tangdu Hospital,
Fourth Military Medical University
China

Dr. Biljana Miljkovic-Selimovic
School of Medicine,
University in Nis,
Serbia; Referent laboratory for Campylobacter and Helicobacter,
Center for Microbiology,
Institute for Public Health, Nis
Serbia

Dr. Xinan Jiao
Yangzhou University
China
Dr. Endang Sri Lestari, MD.  
Department of Clinical Microbiology, Medical Faculty, Diponegoro University/Dr. Kariadi Teaching Hospital, Semarang, Indonesia

Dr. Hojin Shin  
Pusan National University Hospital, South Korea

Dr. Yi Wang  
Center for Vector Biology, 180 Jones Avenue, Rutgers University, New Brunswick, NJ 08901-8536, USA

Dr. Heping Zhang  
The Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, China

Prof. Natasha Potgieter  
University of Venda, South Africa

Dr. Alemzadeh  
Sharif University, Iran

Dr. Sonia Arriaga  
Instituto Potosíno de Investigación Científica Tecnológica/División de Ciencias Ambientales, Mexico

Dr. Armando Gonzalez-Sanchez  
Universidad Autonoma Metropolitana Cuajimalpa, Mexico

Dr. Pradeep Parihar  
Lovely Professional University, Phagwara, Punjab, India

Dr. William H Roldán  
Department of Medical Microbiology, Faculty of Medicine, Peru

Dr. Kanzaki, L I B  
Laboratory of Bioprospection. University of Brasilia, Brazil

Prof. Philippe Dorchie  
Laboratory of Bioprospection. University of Brasilia, Brazil

Dr. C. Ganesh Kumar  
Indian Institute of Chemical Technology, Hyderabad, India

Dr. Farid Che Ghazali  
Universiti Sains Malaysia (USM), Malaysia

Dr. Samira Bouhdid  
Abdelmalek Essaadi University, Tetouan, Morocco

Dr. Zainab Z. Ismail  
Department of Environmental Engineering, University of Baghdad, Iraq

Dr. Ary Fernandes Junior  
Universidade Estadual Paulista (UNESP), Brasil

Dr. Papaevangelou Vassiliki  
Athens University Medical School, Greece

Dr. Fangyou Yu  
The first Affiliated Hospital of Wenzhou Medical College, China

Dr. Galba Maria de Campos Takaki  
Catholic University of Pernambuco, Brazil

Dr. Kwabena Ofori-Kwakye  
Department of Pharmaceutics, Kwame Nkrumah University of Science & Technology, KUMASI, Ghana

Prof. Dr. Liesel Brenda Gende  
Arthropods Laboratory, School of Natural and Exact Sciences, National University of Mar del Plata, Buenos Aires, Argentina.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Adeshina Gbonjubola</td>
<td>Ahmadu Bello University, Zaria, Nigeria</td>
</tr>
<tr>
<td>Prof. Dr. Stylianos Chatzipanagiotou</td>
<td>University of Athens – Medical School Greec</td>
</tr>
<tr>
<td>Dr. Dongqing BAI</td>
<td>Department of Fishery Science, Tianjin Agricultural College, Tianjin 300384 P. R. China</td>
</tr>
<tr>
<td>Dr. Dingqiang Lu</td>
<td>Nanjing University of Technology P.R. China</td>
</tr>
<tr>
<td>Dr. L. B. Sukla</td>
<td>Scientist – G &amp; Head, Biominerals Department, IMMT, Bhubaneswar India</td>
</tr>
<tr>
<td>Dr. Hakan Parlakpinar</td>
<td>MD. Inonu University, Medical Faculty, Department of Pharmacology, Malatya Turkey</td>
</tr>
<tr>
<td>Dr Pak-Lam Yu</td>
<td>Massey University New Zealand</td>
</tr>
<tr>
<td>Dr. Percy Chimwamurombe</td>
<td>University of Namibia Namibia</td>
</tr>
<tr>
<td>Dr. Euclésio Simionatto</td>
<td>State University of Mato Grosso do Sul-UEMS Brazil</td>
</tr>
<tr>
<td>Dr. Hans-Jürg Monstein</td>
<td>Clinical Microbiology, Molecular Biology Laboratory, University Hospital, Faculty of Health Sciences, S-581 85 Linköping Sweden</td>
</tr>
<tr>
<td>Dr. Ajith, T. A</td>
<td>Associate Professor Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur, Kerala-680 555 India</td>
</tr>
<tr>
<td>Dr. Feng-Chia Hsieh</td>
<td>Biopesticides Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture Taiwan</td>
</tr>
<tr>
<td>Prof. Dra. Suzan Pantaroto de Vasconcellos</td>
<td>Universidade Federal de São Paulo Rua Prof. Artur Riedel, 275 Jd. Eldorado, Diadema, SP CEP 09972-270 Brasil</td>
</tr>
<tr>
<td>Dr. Maria Leonor Ribeiro Casimiro Lopes Assad</td>
<td>Universidade Federal de São Carlos - Centro de Ciências Agrárias - CCA/UFSCar Departamento de Recursos Naturais e Proteção Ambiental Rodovia Anhanguera, km 174 - SP-330 Araras - São Paulo Brasil</td>
</tr>
<tr>
<td>Dr. Pierangeli G. Vital</td>
<td>Institute of Biology, College of Science, University of the Philippines Philippines</td>
</tr>
<tr>
<td>Prof. Roland Ndip</td>
<td>University of Fort Hare, Alice South Africa</td>
</tr>
<tr>
<td>Dr. Shawn Carraher</td>
<td>University of Fort Hare, Alice South Africa</td>
</tr>
<tr>
<td>Dr. José Eduardo Marques Pessanha</td>
<td>Observatório de Saúde Urbana de Belo Horizonte/Faculdade de Medicina da Universidade Federal de Minas Gerais Brasil</td>
</tr>
<tr>
<td>Dr. Yuanshu Qian</td>
<td>Department of Pharmacology, Shantou University Medical College China</td>
</tr>
<tr>
<td>Dr. Helen Treichel</td>
<td>URI-Campus de Erechim Brazil</td>
</tr>
</tbody>
</table>
Dr. Xiao-Qing Hu  
*State Key Lab of Food Science and Technology*  
Jiangnan University  
P. R. China

Dr. Olli H. Tuovinen  
*Ohio State University, Columbus, Ohio*  
USA

Prof. Stoyan Groudev  
*University of Mining and Geology “Saint Ivan Rilski”*  
Sofia  
Bulgaria

Dr. G. Thirumurugan  
*Research lab, GLET School of Pharmacy, NH-5, Chaitanya nagar, Rajahmundry-533294.*  
India

Dr. Charu Gomber  
*Thapar University*  
India

Dr. Jan Kuever  
*Bremen Institute for Materials Testing, Department of Microbiology, Paul-Feller-Str. 1, 28199 Bremen*  
Germany

Dr. Nicola S. Flanagan  
*University of Javeriana, Cali*  
Colombia

Dr. André Luiz C. M. de A. Santiago  
*Universidade Federal Rural de Pernambuco*  
Brazil

Dr. Dhruva Kumar Jha  
*Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati 781 014, Assam*  
India

Dr. N Saleem Basha  
*M. Pharm (Pharmaceutical Biotechnology)*  
Eritrea (North East Africa)

Prof. Dr. João Lúcio de Azevedo  
*Dept. Genetics-University of São Paulo-Faculty of Agriculture- Piracicaba, 13400-970*  
Brasil

Dr. Julia Inés Fariña  
*PROIMI-CONICET*  
Argentina

Dr. Yutaka Ito  
*Kyoto University*  
Japan

Dr. Cheruiyot K. Ronald  
*Biomedical Laboratory Technologist*  
Kenya

Prof. Dr. Ata Akcil  
*S. D. University*  
Turkey

Dr. Adhar Manna  
*The University of South Dakota*  
USA

Dr. Cicero Flávio Soares Aragão  
*Federal University of Rio Grande do Norte*  
Brazil

Dr. Gunnar Dahlen  
*Institute of odontology, Sahlgrenska Academy at University of Gothenburg*  
Sweden

Dr. Pankaj Kumar Mishra  
*Vivekananda Institute of Hill Agriculture, (I.C.A.R.), ALMORA-263601, Uttarakhand*  
India

Dr. Benjamas W. Thanomsub  
*Srinakharinwirot University*  
Thailand

Dr. Maria José Borrego  
*National Institute of Health – Department of Infectious Diseases*  
Portugal

Dr. Catherine Carrillo  
*Health Canada, Bureau of Microbial Hazards*  
Canada

Dr. Marcotty Tanguy  
*Institute of Tropical Medicine*  
Belgium
Dr. Han-Bo Zhang
Laboratory of Conservation and Utilization for Bio-
resources
Key Laboratory for Microbial Resources of the
Ministry of Education,
Yunnan University, Kunming 650091.
School of Life Science,
Yunnan University, Kunming,
Yunnan Province 650091.
China

Dr. Ali Mohammed Somily
King Saud University
Saudi Arabia

Dr. Nicole Wolter
National Institute for Communicable Diseases and
University of the Witwatersrand,
Johannesburg
South Africa

Dr. Marco Antonio Nogueira
Universidade Estadual de Londrina
CCB/Depto. De microbiologia
Laboratório de Microbiologia Ambiental
Caixa Postal 6001
86051-980 Londrina.
Brazil

Dr. Bruno Pavoni
Department of Environmental Sciences University of
Venice
Italy

Dr. Shih-Chieh Lee
Da-Yeh University
Taiwan

Dr. Satoru Shimizu
Horonobe Research Institute for the Subsurface
Environment,
Northern Advancement Center for Science &
Technology
Japan

Dr. Tang Ming
College of Forestry, Northwest A&F University,
Yangling
China

Dr. Olga Gortzi
Department of Food Technology, T.E.I. of Larissa
Greece

Dr. Mark Tarnopolsky
Mcmaster University
Canada

Dr. Sami A. Zabin
Al Baha University
Saudi Arabia

Dr. Julia W. Pridgeon
Aquatic Animal Health Research Unit, USDA, ARS
USA

Dr. Lim Yau Yan
Monash University Sunway Campus
Malaysia

Prof. Rosemeire C. L. R. Pietro
Faculdade de Ciências Farmacêuticas de Araraquara,
Univ Estadual Paulista, UNESP
Brazil

Dr. Nazime Mercan Dogan
PAU Faculty of Arts and Science, Denizli
Turkey

Dr Ian Edwin Cock
Biomolecular and Physical Sciences
Griffith University
Australia

Prof. N K Dubey
Banaras Hindu University
India

Dr. S. Hemalatha
Department of Pharmaceutics, Institute of
Technology,
Banaras Hindu University, Varanasi.
221005 India

Dr. J. Santos Garcia A.
Universidad A. de Nuevo Leon
Mexico India

Dr. Somboon Tanasupawat
Department of Biochemistry and Microbiology, Faculty
of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok 10330 Thailand
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Vivekananda Mandal</td>
<td>Post Graduate Department of Botany, Darjeeling Government College, Darjeeling – 734101. India</td>
</tr>
<tr>
<td>Dr. Shihua Wang</td>
<td>College of Life Sciences, Fujian Agriculture and Forestry University China</td>
</tr>
<tr>
<td>Dr. Victor Manuel Fernandes Galhano</td>
<td>CITAB-Centre for Research and Technology of Agro-Environment and Biological Sciences, Integrative Biology and Quality Research Group, University of Trás-os-Montes and Alto Douro, Apartado 1013, 5001-801 Vila Real Portugal</td>
</tr>
<tr>
<td>Dr. Maria Cristina Maldonado</td>
<td>Instituto de Biotecnologia. Universidad Nacional de Tucuman, Argentina</td>
</tr>
<tr>
<td>Dr. Alex Soltermann</td>
<td>Institute for Surgical Pathology, University Hospital Zürich, Switzerland</td>
</tr>
<tr>
<td>Dr. Dagmara Sirova</td>
<td>Department of Ecosystem Biology, Faculty Of Science, University of South Bohemia, Branisovska 37, Ceske Budejovice, 37001 Czech Republic</td>
</tr>
<tr>
<td>Dr. E. O Igbinosa</td>
<td>Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.</td>
</tr>
<tr>
<td>Dr. Hodaka Suzuki</td>
<td>National Institute of Health Sciences, Japan</td>
</tr>
<tr>
<td>Dr. Mick Bosilevac</td>
<td>US Meat Animal Research Center, USA</td>
</tr>
<tr>
<td>Dr. Nora Lia Padola</td>
<td>Imunoquímica y Biotecnología- Fac Cs Vet-UNCPBA, Argentina</td>
</tr>
<tr>
<td>Dr. Maria Madalena Vieira-Pinto</td>
<td>Universidade de Trás-os-Montes e Alto Douro, Portugal</td>
</tr>
<tr>
<td>Dr. Stefano Morandi</td>
<td>CNR-Istituto di Scienze delle Produzioni Alimentari (ISPA), Sez. Milano, Italy</td>
</tr>
<tr>
<td>Dr Line Thorsen</td>
<td>Copenhagen University, Faculty of Life Sciences, Denmark</td>
</tr>
<tr>
<td>Dr. Ana Lucia Falavigna-Guilerme</td>
<td>Universidade Estadual de Maringá, Brazil</td>
</tr>
<tr>
<td>Dr. Baoqiang Liao</td>
<td>Dept. of Chem. Eng., Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, Canada</td>
</tr>
<tr>
<td>Dr. Ou Yang Jinping</td>
<td>Patho-Physiology department, Faculty of Medicine of Wuhan University, China</td>
</tr>
<tr>
<td>Dr. John Sorensen</td>
<td>University of Manitoba, Canada</td>
</tr>
<tr>
<td>Dr. Andrew Williams</td>
<td>University of Oxford, United Kingdom</td>
</tr>
<tr>
<td>Dr. Chi-Chiang Yang</td>
<td>Chung Shan Medical University, Taiwan, R.O.C.</td>
</tr>
<tr>
<td>Dr. Quanming Zou</td>
<td>Department of Clinical Microbiology and Immunology, College of Medical Laboratory, Third Military Medical University, China</td>
</tr>
<tr>
<td>Prof. Ashok Kumar</td>
<td>School of Biotechnology, Banaras Hindu University, Varanasi, India</td>
</tr>
</tbody>
</table>
Dr. Chung-Ming Chen  
*Department of Pediatrics, Taipei Medical University Hospital, Taipei, Taiwan*

Dr. Jennifer Furin  
*Harvard Medical School, USA*

Dr. Julia W. Pridgeon  
*Aquatic Animal Health Research Unit, USDA, ARS, USA*

Dr. Alireza Seidavi  
*Islamic Azad University, Rasht Branch, Iran*

Dr. Thore Rohwerder  
*Helmholtz Centre for Environmental Research UFZ, Germany*

Dr. Daniela Billi  
*University of Rome Tor Vergata, Italy*

Dr. Ivana Karabegovic  
*Faculty of Technology, Leskovac, University of Nis, Serbia*

Dr. Flaviana Andrade Faria  
*IBILCE/UNESP, Brazil*

Prof. Margareth Linde Athayde  
*Federal University of Santa Maria, Brazil*

Dr. Guadalupe Virginia Nevarez Moorillon  
*Universidad Autonoma de Chihuahua, Mexico*

Dr. Tatiana de Sousa Fiuza  
*Federal University of Goias, Brazil*

Dr. Indrani B. Das Sarma  
*Jhulelal Institute of Technology, Nagpur, India*

Dr. Guanghua Wang  
*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, China*

Dr. Renata Vadkertiova  
*Institute of Chemistry, Slovak Academy of Science, Slovakia*

Dr. Charles Hocart  
*The Australian National University, Australia*

Dr. Guoqiang Zhu  
*University of Yangzhou College of Veterinary Medicine, China*

Dr. Guilherme Augusto Marietto Gonçalves  
*São Paulo State University, Brazil*

Dr. Mohammad Ali Faramarzi  
*Tehran University of Medical Sciences, Iran*

Dr. Suppasil Maneerat  
*Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkl University, Hat Yai 90112, Thailand*

Dr. Francisco Javier Las heras Vazquez  
*Almeria University, Spain*

Dr. Cheng-Hsun Chiu  
*Chang Gung memorial Hospital, Chang Gung University, Taiwan*

Dr. Ajay Singh  
*DDU Gorakhpur University, Gorakhpur-273009 (U.P.), India*

Dr. Karabo Shale  
*Central University of Technology, Free State, South Africa*

Dr. Lourdes Zélia Zanoni  
*Department of Pediatrics, School of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil*

Dr. Tulin Askun  
*Balikesir University, Turkey*
Dr. Marija Stankovic  
Institute of Molecular Genetics and Genetic Engineering  
Republic of Serbia

Dr. Scott Weese  
University of Guelph  
Dept of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G2W1, Canada

Dr. Sabiha Essack  
School of Health Sciences  
South African Committee of Health Sciences  
University of KwaZulu-Natal  
Private Bag X54001  
Durban 4000  
South Africa

Dr. Hare Krishna  
Central Institute for Arid Horticulture, Beechwal, Bikaner-334 006, Rajasthan, India

Dr. Anna Mensuali  
Dept. of Life Science, Scuola Superiore Sant’Anna

Dr. Ghada Sameh Hafez Hassan  
Pharmaceutical Chemistry Department, Faculty of Pharmacy, Mansoura University, Egypt

Dr. Kátia Flávia Fernandes  
Biochemistry and Molecular Biology  
Universidade Federal de Goiás  
Brasil

Dr. Abdel-Hady El-Gilany  
Public Health & Community Medicine  
Faculty of Medicine, Mansoura University  
Egypt

Dr. Hongxiong Guo  
STD and HIV/AIDS Control and Prevention, Jiangsu provincial CDC, China

Dr. Konstantina Tsaousi  
Life and Health Sciences, School of Biomedical Sciences, University of Ulster

Dr. Bhavnaben Gowan Gordhan  
DST/NRF Centre of Excellence for Biomedical TB Research  
University of the Witwatersrand and National Health Laboratory Service  
P.O. Box 1038, Johannesburg 2000, South Africa

Dr. Ernest Kuchar  
Pediatric Infectious Diseases, Wroclaw Medical University, Wroclaw Teaching Hospital, Poland

Dr. Hongxiong Guo  
STD and HIV/AIDS Control and Prevention, Jiangsu provincial CDC, China

Dr. Mar Rodriguez Jovita  
Food Hygiene and Safety, Faculty of Veterinary Science. University of Extremadura, Spain

Dr. Jes Gitz Holler  
Hospital Pharmacy, Aalesund. Central Norway Pharmaceutical Trust  
Professor Brochs gt. 6. 7030 Trondheim, Norway

Prof. Chengxiang FANG  
College of Life Sciences, Wuhan University  
Wuhan 430072, P.R.China

Dr. Anchalee Tungtrongchitr  
Sriraj Dust Mite Center for Services and Research  
Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University  
2 Prannok Road, Bangkok Noi, Bangkok, 10700, Thailand
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author’s full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author’s surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the Journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AIMR to publish manuscripts within weeks after submission.

Regular articles
All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer’s name and address. Subheadings should be used. Methods in general use need not be described in detail.
Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors’ experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

Examples:
Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a, b; Tijani, 1993, 1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
**Fees and Charges:** Authors are required to pay a $550 handling fee. Publication of an article in the African Journal of Microbiology Research is not contingent upon the author’s ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

**Copyright: © 2015, Academic Journals.**

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

**Disclaimer of Warranties**

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the AJMR, whether or not advised of the possibility of damage, and on any theory of liability. This publication is provided “as is” without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
ARTICLES

In vitro antibacterial activity of Baillonella toxisperma (Pierre) extracts against Staphylococcus aureus, Salmonella typhi, Proteus mirabilis and Bacillus cereus F3748 2088

Growth and anti-listerial activity of a nisin Z producer in a pork lean meat broth fermentation system 2095
Rosinéa Aparecida de Paula, Ana Andréa Teixeira Barbosa, Sílvia Regina Sartori Machado, Margarete Alice Fontes Saraiva, Célia Alencar de Morais and Hilário C. Mantovani
In vitro antibacterial activity of *Baillonella toxisperma* (Pierre) extracts against *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* F3748

S. H. Riwom Essama¹*, J. Ngo Mbing², M. A. Nyegue¹, C. Ndoye Foe², M. Fodouop¹, S. P. Bouopda Tamo¹, D. E Pegnyemb³ and F. X. Etoa¹

¹Laboratory of Microbiology, P. O. Box 812 Yaoundé, Cameroon.
²Laboratory of Phytobiochemistry and Medicinal Plant Study, P. O. Box 812 Yaoundé, Cameroon.
³Laboratory of Chemical Pharmacology and Natural Substances P. O. Box 812 Yaoundé, Cameroon.

Received 7 July, 2015; Accepted 31 August, 2015

This study evaluated the *in vitro* antibacterial activity of the ethyl acetate, acetone, methanol and hydroethanol mixture (2:8) extracts of the leaves and stem-barks of *Baillonella toxisperma* (Pierre), harvested in the East and center regions of Cameroon, on *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* F3748. These bacteria are usually responsible for diarrheal diseases and in severe cases can lead to the death of patients. The susceptibility of the bacteria to the extracts was evaluated by the well diffusion method and the inhibition parameters of the bacterial growth were determined by the micro-dilution assay according to the directives of document M27-A9 (2012) of the Clinical and Laboratory Standards Institutes (CLSI). The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal concentrations (MBC) obtained were between 1.56 and 25.00 mg/ml. Stem-barks ethyl acetate extract from the East region, was most active on *S. aureus*, *S. typhi* and *P. mirabilis* with a MBC of 6.25 mg/ml. The leaves methanolic extracts from the center region was the most active with a MBC of 6.25 mg/ml on *S. aureus*. The ratio MBC/MIC shows that the majority of the extracts were bacteriostatic on the strains tested. The phytochemical screening revealed that the plant contained bioactive substances such as phenols, tannins, flavonoids, steroids, alkaloids, saponins, triterpenes and cardiac glycosides, reported by several authors for their antibacterial activity. The results obtained validate the traditional use of this plant in the treatment of affections of bacterial origin.

Key words: Cameroon, *Baillonella toxisperma* (Pierre), bioactive substances, antibacterial activity.

INTRODUCTION

Bacterial infections constitute a serious public health problem in the world. Among the causative agents, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus cereus* are cited in most clinical cases. *S. aureus* is one of the principal causes of food toxic-infections which are characterized by a severe appearance of diarrhoea. It is the almost-universal cause of furuncles, carbuncles, and skin abscesses and worldwide is the most commonly identified agent responsible for skin and soft tissue infections (McCaig et al., 2006). *S. typhi* is the causative agent of typhoid fever and diarrhoea. *B. cereus* is responsible for food poisoning and diarrheal syndromes...
(Logan and Rodriguez-Diaz, 2006). *P. mirabilis* on its part is responsible for urinary tract, cutaneous, respiratory tract infections, septicaemia and bacteremia (Ronald, 2003). These bacterial infections can in extreme cases, lead to the death of the patient. Moreover, these bacteria have over time developed resistance to certain usual antibiotics. It is the case of *B. cereus* which has become resistant to penicillin, ampicillin, cephalosporines and trimethoprim (Murray et al., 2007). There is an imperative need for the research and renewal of active ingredients which have become ineffective due to the emergence of the phenomenon of microbial multi-resistance to common antibiotics.

Medicinal plants via their secondary metabolites constitute a potential source of antimicrobial (Li et al., 2007). Many scientific studies have been undertaken in order to study the botanical and therapeutic aspects of the latter and to integrate their medicinal properties in a modern health system by exploiting their active ingredients (Bijiti et al., 2004). Many bioactive compounds isolated from plants such as flavonoids, phenolic alkaloids, saponins, tannins, coumarins, phenolic acids and terpenes, were been used for a long time as active ingredients in the development of anti-infectious drugs (Ghost et al., 2007).

*Baillonella toxisperma* (Pierre) is a plant of the Cameroonian pharmacopeia, traditional hailed for its medicinal virtues. Commonly called Moabi, this plant is used traditionally to treat infectious drugs (Ghost et al., 2007).

Many scientific studies have been undertaken in order to study the botanical and therapeutic aspects of the latter and to integrate their medicinal properties in a modern health system by exploiting their active ingredients (Bijiti et al., 2004). Many bioactive compounds isolated from plants such as flavonoids, phenolic alkaloids, saponins, tannins, coumarins, phenolic acids and terpenes, were been used for a long time as active ingredients in the development of anti-infectious drugs (Ghost et al., 2007).

*Baillonella toxisperma* (Pierre) is a plant of the Cameroonian pharmacopeia, traditional hailed for its medicinal virtues. Commonly called Moabi, this plant is used traditionally to treat infectious drugs (Ghost et al., 2007).

Many scientific studies have been undertaken in order to study the botanical and therapeutic aspects of the latter and to integrate their medicinal properties in a modern health system by exploiting their active ingredients (Bijiti et al., 2004). Many bioactive compounds isolated from plants such as flavonoids, phenolic alkaloids, saponins, tannins, coumarins, phenolic acids and terpenes, were been used for a long time as active ingredients in the development of anti-infectious drugs (Ghost et al., 2007).

The preliminary tests of sensitivity of the bacterial strains to the various extracts were carried out as recommended by CLSI (2005). 100 µl of each bacterial inoculum was inoculated on Mueller Hinton agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).

**Preparation of the bacterial inoculum**

For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).

**Preparation of the leaves and stem-barks extracts of Baillonella toxisperma (Pierre)**

Extracts of the botanical material were extracted according to the protocol described by Prakash and Gupta (2005). The leaves and stem-barks were cut out into scraps then, dried at ambient temperature, free from moisture and light. The dried plant materials were finely crushed using an electric blender. The powder obtained was macerated in four solvents: ethyl acetate, acetone, methanol and ethanol-water (8: 2). 100 g of powdered stem-barks and leaves were macerated in 500 ml of each solvent for 48 h. The marcs obtained were filtered through Whatman N° 1 filter paper and the filtrates collected in conical flasks. This process was repeated thrice for complete exhaustion of the plant material and the filtrates obtained were concentrated in a rotavapor. The dry extracts were preserved at +4°C in a refrigerator. The extraction yields expressed in percentage (%) were determined by the formula below:

\[
\text{Yield} (\%) = \left( \frac{\text{Mass of macerated powder}}{\text{Mass of the extract}} \right) \times 100
\]

**Phytochemical screening**

Determination of the phytochemical composition of the various extracts was carried out according to standard methods described by Harbone (1998) and Sofowora (1993).

**Determination of the inhibition parameters: MIC and MBC**

The inhibition parameters of bacterial growth were evaluated according to the M27-A9 guideline described by CLSI (2012). This involved preparing double dilutions of tested substances in 100 µl of glucose supplemented nutrient broth (GNB) medium (Acumedia Manufacturers) into the wells of a microtiter. The range of final concentrations was prepared using a microplate diluter. For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).

**Preparation of the bacterial inoculum**

For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).

**Preparation of the leaves and stem-barks extracts of Baillonella toxisperma (Pierre)**

Extracts of the botanical material were extracted according to the protocol described by Prakash and Gupta (2005). The leaves and stem-barks were cut out into scraps then, dried at ambient temperature, free from moisture and light. The dried plant materials were finely crushed using an electric blender. The powder obtained was macerated in four solvents: ethyl acetate, acetone, methanol and ethanol-water (8: 2). 100 g of powdered stem-barks and leaves were macerated in 500 ml of each solvent for 48 h. The marcs obtained were filtered through Whatman N° 1 filter paper and the filtrates collected in conical flasks. This process was repeated thrice for complete exhaustion of the plant material and the filtrates obtained were concentrated in a rotavapor. The dry extracts were preserved at +4°C in a refrigerator. The extraction yields expressed in percentage (%) were determined by the formula below:

\[
\text{Yield} (\%) = \left( \frac{\text{Mass of macerated powder}}{\text{Mass of the extract}} \right) \times 100
\]

**Materials and Methods**

Preparation of the leaves and stem-barks extracts of *Baillonella toxisperma* (Pierre)

Extracts of the botanical material were extracted according to the protocol described by Prakash and Gupta (2005). The leaves and stem-barks were cut out into scraps then, dried at ambient temperature, free from moisture and light. The dried plant materials were finely crushed using an electric blender. The powder obtained was macerated in four solvents: ethyl acetate, acetone, methanol and ethanol-water (8: 2). 100 g of powdered stem-barks and leaves were macerated in 500 ml of each solvent for 48 h. The marcs obtained were filtered through Whatman N° 1 filter paper and the filtrates collected in conical flasks. This process was repeated thrice for complete exhaustion of the plant material and the filtrates obtained were concentrated in a rotavapor. The dry extracts were preserved at +4°C in a refrigerator. The extraction yields expressed in percentage (%) were determined by the formula below:

\[
\text{Yield} (\%) = \left( \frac{\text{Mass of macerated powder}}{\text{Mass of the extract}} \right) \times 100
\]

**Phytochemical screening**

Determination of the phytochemical composition of the various extracts was carried out according to standard methods described by Harbone (1998) and Sofowora (1993).

**Preparation of the bacterial inoculum**

For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).

**Preliminary sensitivity test of the strains to the extracts**

The preliminary tests of sensitivity of the bacterial strains to the various extracts were carried out as recommended by CLSI (2005). 100 µl of each bacterial inoculum was inoculated on Mueller Hinton agar (Fortress Diagnostics Limited U.K). The Petri dishes were then allowed to dry at ambient temperature under a fumes cupboard for 15 min. 6 mm wells were bored in the agar and the bottom of each well plugged with a drop of Mueller Hinton agar to limit the diffusion of the extracts from below. Fixed volumes of 50 µl of the stock solutions of the extracts (50 mg/ml) and gentamicin (1 mg/ml) were then introduced into each well. After a pre diffusion time of 15 min of the antibacterial substances to be tested at ambient temperature, the Petri dishes were incubated at 37°C for 24 h. The inhibition diameters round each well was measured using a sliding caliper. Each test was carried out in triplicate and the inhibition diameters expressed mean ± standard deviation.

**Determination of the inhibition parameters: MIC and MBC**

The inhibition parameters of bacterial growth were evaluated according to the M27-A9 guideline described by CLSI (2012). This involved preparing double dilutions of tested substances in 100 µl of glucose supplemented nutrient broth (GNB) medium (Acumedia Manufacturers) into the wells of a microtiter. The range of final concentrations was prepared using a microplate diluter. For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 10⁶ CFU/mL (0.5 McFarland). After a 10⁸ dilution, the bacterial load was 10⁶ CFU/ml (Hernandez et al., 2000).
concentrations tested were 25 to 0.097 mg/ml for each plant extract and 0.250 to 0.00097 mg/ml for gentamicin (Brunhild Pharmaceutical Private Limited). Each serial dilution was performed in triplicate. The bacterial inoculum was prepared at 10⁶ CFU/mL using McFarland. Volumes of 100 µL of this inoculum were distributed to all the wells of the microtiter. A line of the plate without plant extract served as a control for the growth of the organism (negative control) and another (without plant extract and without inoculum) served as sterility testing medium (positive control). The microtitre plates were thereafter sealed with aluminum foil and incubated at 37°C for 24 h. After incubation, 40 µl of 2,3,5-triphenyl tetrazolium chloride (Sigma-Aldrich) (0.2 mg/mL) were introduced into each well (Burdock et al., 2011). The MIC was the smallest concentration for fresh GNB. The microtitre plates were incubated for 48h at 37°C, thereafter revealed as earlier done. The smallest concentration for which the medium to red. The MBC were determined by subculture. 50 µL of the contents of wells greater than or equal to the MIC was introduced into 150 µL of fresh GNB. The microtitre plates were incubated for 48h at 37°C, thereafter revealed as earlier done. The smallest concentration for which no color change was observed was regarded as the minimum bactericidal concentration.

RESULTS

Extraction yield

The extraction yield of the leaves and stem-barks of *B. toxisperma* (Pierre) are shown in Table 1. It is observed that the extraction yields are comprised between 1.08% (stem-barks ethyl acetate) and 10.07% (leaves hydro-ethanolic extract) for the plant material harvested in the Center and between 1.53% (stem-barks ethyl acetate) and 18.34% (hydro-ethanolic stem-barks) for the plant material harvested in the East region.

### Phytochemical screening

The phytochemical screening revealed the presence of several groups of secondary metabolites such as tannins, the flavonoids, steroids, saponins, terpenoids and phenols in both extracts of *Baillonella toxisperma* (Pierre) harvested from the East and Center regions of Cameroon. Table 2 summarizes the results obtained from the screening depending on the extracts considered.

### Susceptibility test

The results obtained from the susceptibility test (Table 3) show that the bacterial strains were sensitive to the leaves and stem-barks extracts of *B. toxisperma* (Pierre). For the plant material harvested in the East, the inhibition diameters of the leaves crude extracts was between 8.66 ± 0.57 mm (hydro-ethanolic extract on *B. cereus*) and 11.33 ± 0.57 mm (methanolic extract on *B. cereus*), and for the botanical material harvested in the Center, the inhibition diameters ranged from 9.00 ± 1.00 mm (acetone extract) to 11.66 ± 0.57 mm (methanolic extract on *S. typhi*). The inhibition diameters of the stem-barks crude extracts was comprised between 9.66 ± 0.57 mm (hydro-ethanolic extract on *S. aureus*) and 19.66 ± 0.57 mm

---

Table 1. Extraction yields and physico-chemical characteristics of leaves and stem-barks extracts of *Baillonella toxisperma* (Pierre).

<table>
<thead>
<tr>
<th>Sites of harvest</th>
<th>Extraction Solvents</th>
<th>Parts of the plant</th>
<th>Extraction yield (%)</th>
<th>Physical properties of the extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Color</td>
</tr>
<tr>
<td>Dimako</td>
<td>Hexane</td>
<td>Stem-barks</td>
<td>2.42</td>
<td>Yellow</td>
</tr>
<tr>
<td>(East Region - Cameroon)</td>
<td>Ethyl-acetate</td>
<td>Leaves</td>
<td>2.59</td>
<td>Dark-green</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>Stem-barks</td>
<td>1.53</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td>Ethanol-water (8/2)</td>
<td>Leaves</td>
<td>6.24</td>
<td>Dark-green</td>
</tr>
<tr>
<td>21/01/15</td>
<td>Acetone</td>
<td>Stem-barks</td>
<td>14.02</td>
<td>Thick-red</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>8.88</td>
<td>Dark-green</td>
</tr>
<tr>
<td>Mbalmaya</td>
<td>Hexane</td>
<td>Stem-barks</td>
<td>7.92</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>(Center Region - Cameroon)</td>
<td>Ethyl-acetate</td>
<td>Leaves</td>
<td>13.95</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>Stem-barks</td>
<td>8.32</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>Ethanol-water (8/2)</td>
<td>Leaves</td>
<td>6.80</td>
<td>Dark-green</td>
</tr>
<tr>
<td>Le 09/12/14</td>
<td>Acetone</td>
<td>Stem-barks</td>
<td>2.75</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>3.16</td>
<td>Dark-green</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stem-barks</td>
<td>1.08</td>
<td>Yellowish</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>2.40</td>
<td>Dark-green</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stem-barks</td>
<td>4.72</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>6.85</td>
<td>Thick-red</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>10.7</td>
<td>Greenish</td>
</tr>
</tbody>
</table>

---

Table 2. Phytochemical screening.

<table>
<thead>
<tr>
<th>Phytochemical groups</th>
<th>Plant extract</th>
<th>East-region</th>
<th>Center-region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E1</td>
<td>F1</td>
<td>E2</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tanins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponines</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiacglycosids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

E1 and F1, Acetone and ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves from the East; E3 and F3, hydro-ethanolic stem-barks and leaves from the East; E4 and F4, acetone stem-barks and leaves extracts from the East; E’1 and F’1, ethyl acetate stem-barks and leaves from the Center; E’2 and F’2, Methanol stem-barks and leaves extract from the Center; E’3 and F’3, Hydro-ethanolic extracts from the Center; E’4 and F’4, Acetone stem-barks and leaves from the Center; +, Presence of compound; -, absence of compound.

(acetone extract on S. typhi) for the plant material harvested in the East and between 7.33± 0.57 mm (ethyl acetate and acetone extracts on S. aureus and P. mirabilis) and 17.00 ± 0.00 mm (acetone extract on S. typhi) for the plant material harvested in the Center.

Determination of the inhibition parameters

The results obtained for the inhibition parameters (Table 4) show that the MIC of the plant material from the East was comprised between 1.56 and 25 mg/ml and that of the Center, between 3.12 and 25 mg/ml. The MBC was between 6.25 and 25 mg/ml. According to Fauchère and Avril (2002) when the MBC of an antibiotic on a given strain is close to the MIC (1 ≤ MBC/MIC ≤ 2), the antibiotic is described as being bactericidal. On the other hand, when these values are relatively distant, (4 ≤ MBC/MIC ≤ 16), the antibiotic is known to be bacteriostatic. Lastly if the MBC/MIC >16, it is described tolerant.

DISCUSSION

The extraction yields of the leaves and barks show that the extraction yields (Table 1) were between 1.08% (ethyl acetone stem-barks extracts) and 10.07 % (hydroethanolic leaves extracts) for the plant material from the Center region and between 1.53 % (ethyl acetate stem-barks extracts) and 18.34% (Stem-barks ethanol-water extract) for the plant material collected in the East region. For the same solvent and plant organ (leaves or bark), variations in the extraction yields could be due to edaphic and climatic factors. Globally, methanol and hydro-ethanol extracts gave the best extraction yields. This could be explained by the fact that the secondary metabolites extracted are more soluble in alcohols (Bruneton, 1999).

Results obtained from the phytochemical screening (Table 2) of the extracts of B. toxisperma (Pierre) show that this plant is endowed with secondary metabolites such as phenols, saponins, tannins, flavonoids, triterpenes, steroids and cardiac glycosids. These bioactives substances have been reported by several authors for their antibacterial activity. These bioactive compounds have long been used in modern medicine for drug development (Dawang and Datup, 2012). Several molecules isolated from plants such as pinocebrine, ponciretine, sophora flavanone G and naringine significantly showed antimicrobial activities in both Gram positive and Gram negative bacteria (Tim and Andrew, 2005). For the same solvent and the same plant organ (leaves or bark), variations in the phytochemical composition were observed. This could be due to ecological parameters, which generally differ from one area to another depending on geographic distance. These differences can strongly influence the biology and the physiology of the plants, in particular their composition in secondary metabolites (Etchikè et al., 2011).

The results obtained from the susceptibility test (Table 3) show that at a concentration of 50 mg / ml, the inhibition diameters of the bacterial growth were between 6.66 ± 0.57 and 19.66 ± 0.57 mm. For a given strain, these inhibition diameters were however lower than those of gentamicin (13.00 ± 0.00 to 28.66 ± 0.57 mm). The distinct sensitivity of the strains with regards to the extracts could be due to the intrinsic features specific to each micro-organism (permeability of the cell wall, presence of an external membrane) and with the phytochemical profile of the extracts (Takeo et al., 2004; Achrif et al., 2012). The Gram positive bacteria (S. aureus and B. cereus) were more sensitive to the toxic effect of the extracts than their Gram negative (S. typhi).
of the membrane of Gram negative bacteria (Duffy and Power, 2001). The surface of Gram-negative bacteria is largely composed of the glycolipid lipopolysaccharide (LPS), serving as one of the initial barriers against extracellular stresses. Specifically, LPS is a major constituent of the outer leaflet of the outer membrane phospholipid bilayer, which envelops the peptidoglycan containing periplasm and the inner membrane (Band and Weiss, 2015).

With regards to the inhibition parameters (Table 3), the MIC ranges from 1.56 to 25.00 mg/ml, and the MBC between 6.25 to 25.00 mg/ml. The ratio MBC/MIC was determined and according to the classification made by Fauchère and Avril (2002), the extracts presented an antibacterial action on most of the strains (4 ≤ CMB/CMI ≤ 16) except acetone and hydro-ethanolic extracts which were bactericidal.

Table 3. Susceptibility test.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>Gen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>15.33±</td>
<td>16.66±</td>
<td>11.33±</td>
<td>16.00±</td>
<td>8.66±</td>
<td>16.00±</td>
<td>10.33±</td>
<td>13.33±</td>
<td>14.00±</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12.00±</td>
<td>10.33±</td>
<td>9.66</td>
<td>10.00±</td>
<td>10.33±</td>
<td>6.66±</td>
<td>7.33±</td>
<td>10.33±</td>
<td>13.00±</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>18.66±</td>
<td>18.00±</td>
<td>11.00±</td>
<td>19.00±</td>
<td>19.66±</td>
<td>11.00±</td>
<td>11.00±</td>
<td>13.66±</td>
<td>11.66±</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>15.00±</td>
<td>10.33±</td>
<td>14.00±</td>
<td>14.33±</td>
<td>7.33±</td>
<td>10.66±</td>
<td>10.33±</td>
<td>10.00±</td>
<td>15.00±</td>
</tr>
</tbody>
</table>

E1 and F1, Ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves extracts from the East; E3 and F3, Hydro-ethanolic stem-barks and leaves extracts from the East; E4 and F4, Acetone stem-barks and leaves extracts from the East; E1 and F1, Ethyl acetate stem-barks and leaves extracts from the Center; E2 and F2, Methanol stem-barks and leaves extracts from the Center; E3 and F3, Hydro-ethanolic stem-barks and leaves extracts from the Center; E4 and F4, Acetone stem-barks and leaves extracts from the Center; Hu, Oil; Gen, Gentamicin.

and P. mirabilis counterparts. This could be due to the significant differences in the outer layer of Gram positive and Gram negative bacteria. Gram negative bacteria possess an external membrane and a periplasmic space which is absent in Gram positive bacteria (Duffy and Power, 2001). The surface of Gram-negative bacteria is largely composed of the glycolipid lipopolysaccharide (LPS), serving as one of the initial barriers against extracellular stresses. Specifically, LPS is a major constituent of the outer leaflet of the outer membrane phospholipid bilayer, which envelops the peptidoglycan containing periplasm and the inner membrane (Band and Weiss, 2015).

With regards to the inhibition parameters (Table 3), the MIC ranges from 1.56 to 25.00 mg/ml, and the MBC between 6.25 to 25.00 mg/ml. The ratio MBC/MIC was determined and according to the classification made by Fauchère and Avril (2002), the extracts presented an antibacterial action on most of the strains (4 ≤ CMB/CMI ≤ 16) except acetone and hydro-ethanolic extracts which were bactericidal.

Based on the in vitro antibacterial activity obtained with the extracts of B. toxisperma (Pierre), this plant englobes a set of criteria which could justify the renewed interest for the exploitation of this natural resource in the development of antibacterial drugs order to mitigate the narrow activity spectrum which the usual molecules pose.

Conclusion

The results obtained in this study bring scientific justification as to the use of B. toxisperma (Pierre) in traditional medicine for the treatment of microbial infections, in particular those of bacterial origin. Depending on the harvesting site of the plant (East and Center regions of Cameroon), more or less significant variations in the antibacterial activity was observed. These variations were attributed to edaphic and climatic factors which influenced the qualitative and quantitative chemical composition of the secondary metabolites in the plant at their site of growth. Phytochemical
Table 4. Inhibition parameters: MIC, MBC, MBC/MIC.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Inhibition parameter (mg/ml)</th>
<th>Plant extracts</th>
<th>Center-region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC</td>
<td>1.5</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>MBC</td>
<td>12</td>
<td>ND</td>
<td>12</td>
</tr>
<tr>
<td>MBC/MIC</td>
<td>8</td>
<td>ND</td>
<td>8</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC</td>
<td>1.5</td>
<td>6.2</td>
<td>3.1</td>
</tr>
<tr>
<td>MBC</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>MBC/MIC</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC</td>
<td>1.5</td>
<td>6.2</td>
<td>1.5</td>
</tr>
<tr>
<td>MBC</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>MBC/MIC</td>
<td>4</td>
<td>ND</td>
<td>8</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC</td>
<td>1.5</td>
<td>6.2</td>
<td>1.5</td>
</tr>
<tr>
<td>MBC</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>MBC/MIC</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

E1 and F1, Ethyl acetate stem-barks and leaves from the East; E2 and F2, Methanol stem-barks and leaves extracts from the East; E3 and F3, Hydro-ethanolic stem-barks and leaves from the East; E4 and F4, Acetone stem-barks and leaves extracts from the East; E'1 and F'1, Ethyl acetate stem-barks and leaves extracts from the Center; E'2 and F'2, Methanol stem-barks and leaves extracts from the Center; E'3 and F'3, Hydro-ethanolic stem-barks and leaves extracts from the Center; E'4 and F'4, Acetone stem-barks and leaves extracts from the Center; Hu, Oil; Gen, Gentamicin; ND, not determined.

Screening of the extracts of the plant material from the East region and that from the Center region showed that the two samples were rich in terpenoids, tannins, flavonoids, phenols, saponins, steroids and cardiac glycosides. These bioactive molecules can be isolated from this plant and used in the development of pharmaceutical specialties capable of ensuring the treatment of many infectious diseases.

**Conflict of interests**

The author(s) did not declare any conflict of interest.

**ACKNOWLEDGEMENTS**

The authors thank the University of Yaoundé I for providing the framework, “Centre Pasteur” of Cameroon for having provided us with the bacteria strains used in this work and the National Herbarium of Yaoundé-Cameroon for botanical identification of *B. toxisperma* (Pierre).

**REFERENCES**


Growth and anti-listerial activity of a nisin Z producer in a pork lean meat broth fermentation system

Rosinéa Aparecida de Paula¹, Ana Andréa Teixeira Barbosa¹², Silvia Regina Sartori Machado¹, Margarete Alice Fontes Saraiva¹, Célia Alencar de Moraes¹ and Hilário C. Mantovani¹*

¹Universidade Federal de Viçosa, Departamento de Microbiologia, Viçosa, Minas Gerais, 36570-900, Brazil.
²Universidade Federal de Sergipe, Departamento de Morfologia, São Cristóvão, Sergipe, Brazil.

Received 9 May, 2015; Accepted 6 July, 2015

Listeria monocytogenes is widely distributed in nature and has been isolated from numerous sources such as meat and fermented meat products. This pathogenic microorganism can resist conditions of low pH, low water activity (Aw), high salt (NaCl) concentrations and the presence of sodium nitrite, being able to survive the commercial sausage manufacturing process. The aim of this work was to evaluate the antilisterial activity of a lactic acid bacterium (Lactococcus lactis subsp. lactis PD 6.9) isolated from Italian-style salami in conditions that simulate salami fermentation. L. lactis PD 6.9 produces nisin Z and grows well in pork lean meat broth, a feature that would be useful to compete with food-borne pathogens. The peak of nisin Z production by L. lactis PD 6.9 in pork lean meat broth occurred after 14 h of fermentation, but the inhibitory activity decreased if the producer organism was maintained in stationary phase. When L. lactis PD 6.9 (10⁷ CFU ml⁻¹) and Listeria monocytogenes LMA 20 (10⁶ CFU ml⁻¹) were co-inoculated in pork lean meat broth, growth of L. monocytogenes was unaffected. The decrease in viable cell number of Listeria coincided with an increase in bacteriocin activity produced by L. lactis PD 6.9 in pork lean meat broth. Co-culture experiments indicated that L. lactis PD 6.9 was able to control the growth of L. monocytogenes even if the Listeria population was 1000-fold greater than the L. lactis population. These results demonstrate the potential application of L. lactis PD 6.9 in controlling the growth of L. monocytogenes during salami fermentation and its usefulness as a starter culture for fermented sausages.

Keywords: Listeria monocytogenes, bacteriocins, lactic acid bacteria, co-cultivation, salami.

INTRODUCTION

Fermented sausages are produced by fermentation of minced meat mixed with fat, salt, curing agents (nitrate/nitrite), sugars and spices (Caplice and Fitzgerald, 1999). In order to speed the process and ensure the quality and uniformity of the final product, lactic acid bacteria (LAB) are commonly used as starter...
cultures to decrease the pH (Marchesini et al., 1992; Liu et al., 2010). These LAB produce organic acids that enhance the aroma and extend the shelf life of the fermented product. However, the hurdles faced by microorganisms during sausage fermentation may not prevent the survival of pathogenic bacteria, including Clostridium, Escherichia coli O157:H7 and Listeria (Bonnet and Montville, 2005; Mor-Mur and Yuste, 2010; Martin et al., 2011; Hospital et al., 2012).

Listeria monocytogenes is a gram-positive food-borne pathogen widely distributed in nature and has been isolated from numerous sources such as meat and fermented meat products, including fermented sausages (Martin et al., 2011; Martins and Germano, 2011; Meloni et al., 2014). L. monocytogenes can resist conditions of low pH, low water activity (Aw), high salt (NaCl) concentrations and the presence of sodium nitrite, and is able to survive the commercial sausage manufacturing process (Bonnet and Montville, 2005; Degenhardt and Sant’Anna, 2007). The ingestion of food products contaminated with L. monocytogenes is particularly dangerous to young, old, pregnant and immune compromised individuals (Tauxe, 2002; Thévenot et al., 2005; Barlik et al., 2014) and even small number of Listeria appear capable to causing disease.

Bacteriocin-producing starter cultures appears to be an effective strategy to control Listeria in fermented sausages (Gormley et al., 2010; Freitas de Macedo et al., 2011; Rubio et al., 2014). Lactococcus lactis subsp. lactis PD 6.9, a lactic acid bacterium isolated from Italian salami (Maciel et al., 2003), was previously shown to inhibit the growth of L. monocytogenes in liquid culture and the antimicrobial activity was due to bacteriocin production (Carvalho et al., 2006). Further work indicated that L. lactis PD 6.9 harbor the gene encoding nisin Z on the chromosome and purification of the peptide from the cell free supernatant followed by mass spectrometry analysis confirmed the molecular mass to be 3329.57 Da (Saraiva et al., 2014). Although L. lactis PD 6.9 showed great potential to inhibit foodborne pathogens, the production and activity of this bacteriocin had not yet been demonstrated in a liquid model system that simulates the conditions found during salami fermentation. Therefore, we hypothesized that L. lactis PD 6.9 could be useful as a starter culture for fermented sausages.

The aim of the present work was to examine the ability of L. lactis PD 6.9 to grow and produce bacteriocin in conditions that simulate the fermentation of minced meat and thus to verify its possible antagonism against L. monocytogenes in such medium.

**MATERIALS AND METHODS**

**Microorganisms and growth conditions**

L. lactis subsp. lactis PD 6.9, producer of nisin Z, was previously isolated from Italian salami processed by natural fermentation (Maciel et al., 2003), and kept stored at −80°C in D-MRS (Modified deMan, Rogosa e Sharpe media) (Hammes et al., 1992) supplemented with 20% glycerol. Before use, the culture was activated three times in D-MRS at 30°C.

L. monocytogenes LMA 20, isolated from chicken carcass, was obtained from the culture collection of the Food Microbiology Laboratory at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (Carvalho et al., 2006). It was routinely transferred in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and incubated at 37°C. Stock cultures were grown in the same medium and stored at −80°C.

**Growth in a liquid model system that simulates the conditions found during minced meat fermentation**

Portions of pork fresh lean meat (250 g) were obtained from a local abattoir, aseptically minced, placed into a blender (Walita Beta, São Paulo, Brazil) and mixed with approximately 200 ml of a 0.85% NaCl solution. The volume was adjusted to 500 mL and the mixture was centrifuged twice at 10,000 x g for 10 min at 5°C. The supernatant was collected and supplemented with 1.0% glucose, 3.0% NaCl, 120 ppm NaN3O, 200 ppm NaN3. The pork lean meat broth was filtered through nitrocellulose membranes of 0.45 µm pore size (Schleicher and Schuell BioScience, Keene, USA) and stored at 4°C until use.

Cultures of L. lactis PD 6.9, were subcultured in pork lean meat broth (105 CFU ml−1) and incubated at 25°C under aerobic conditions. Samples (100 µl) were taken at different time intervals. Colony forming units per milliliter (CFU ml−1) at each time interval were determined after 24 h of incubation. L. monocytogenes LMA 20 was inoculated into pork lean meat broth (106 to 107 CFU ml−1), and incubated at 25°C under aerobic conditions. Growth was monitored by enumeration of the viable cell number for up to 32 h on tryptone soy agar supplemented with yeast extract (TSAYE) and incubated at 37°C. The experiments were performed at least in duplicate and the results represent the average from two independent observations. The specific growth rate (µ) of cultures was estimated from the rate of increase in cell number (X) based on the equation dX/dt = µX, where µ is an absolute rate constant with units of h−1 and t is the growth time in exponential phase.

**Antimicrobial activity in pork lean meat broth**

L. lactis PD 6.9 was grown in pork lean meat broth to stationary phase. Inhibitory activity and pH were monitored over time. Aliquots (1 ml) of the cultures were centrifuged at 10,000 x g for 5 min, the supernatants were collected, and the pH was measured using an Accumet® model 15 pHmeter (Fisher Scientific, Pittsburgh, USA). The supernatants were separated to pH 6.5 with NaOH 5 M, filtered in nitrocellulose membranes of 0.22 µm pore size (Schleicher and Schuell BioScience, Keene, USA) and tested for bacteriocin activity against L. monocytogenes LMA 20 by the agar well diffusion assay (Tagg et al., 1976).

Co-culture experiments in pork lean meat broth were carried out in batch cultures inoculated with 104 to 107 CFU ml−1 of L. monocytogenes LMA 20 and 106 to 107 CFU ml−1 of L. lactis PD 6.9, as indicated in the Figure legends. Samples of the co-cultures were taken at different time intervals and serially diluted (10-fold dilutions) into sterile saline solution (0.85% NaCl). Aliquots (20 µl) were plated onto D-MRS containing CaCO3 (5 g l−1) and bromocresol purple (0.04 g l−1) for viable counts of L. lactis PD 6.9. L monocytogenes was enumerated in TSAYE media supplemented with 1.5% lithium chloride after 48 h of incubation. Culture pH was determined as described above. Co-culture experiments were also performed using a no-bacteriocinogenic strain, Lactococcus lactis
ATCC 19435. *L. monocytogenes* (10^7 CFU ml^-1) and *L. lactis* (10^7 CFU ml^-1) were co-inoculated in pork lean meat broth and the viable cell number was monitored up to 48 h as described above. The experiments were performed at least in duplicate and the results represent the mean from two independent observations. Enumeration of *L. monocytogenes* was determined from triplicate plate counts of each dilution.

**Experimental design and statistics**

The experiments were performed in two biological replicates. To evaluate the growth of *Listeria monocytogenes* and *Lactococcus lactis* PD 6.9 in pork lean meat broth, two samples for each time point were harvested for enumeration. To evaluate the antagonistic activity pork lean meat broth, triplicate plate counts were prepared for each dilution and for each biological replicate. The error bars in figures indicate the standard deviation of the mean.

**RESULTS AND DISCUSSION**

**Growth of *L. lactis* PD 6.9 and *L. monocytogenes* in pork lean meat broth**

In this study, we tested the antagonistic properties of a nisin Z-producing strain of *L. lactis* that was previously isolated from naturally fermented Italian salami. When *L. lactis* PD 6.9 was inoculated (approximately 10^7 CFU ml^-1) in pork lean meat broth, the growth rate was 0.59 h^-1 and the culture reached stationary phase after approximately 8 h of incubation (Figure 1a). The pH of the media decreased rapidly after 6 h of incubation and was as low as 4.2 at the end of the experiment. Bacteriocin activity was generally detected when the cell population was greater than 10^8 CFU ml^-1, and the maximum inhibitory activity occurred after 14 h of incubation (Figure 1b).

Bacteriocin purification and DNA sequencing demonstrated that the peptide produced by *L. lactis* PD 6.9 is a natural nisin A variant, nisin Z, as indicated by the substitution of a histidine by an asparagine residue at position 27 of the bacteriocin sequence (Saraiva et al., 2014). Nisin A and nisin Z appear to have similar biological activity, but often differ in their physicochemical properties, such as the diffusion is solid matrices.

In this study, the activity of nisin Z in the cell-free supernatants decreased after 14 h of fermentation, and inhibition zones could not be detected if cultures were maintained in stationary phase for more than 16 h. However, it should be noted that the same observation was done when the bacterium was cultivated alone in D-MRS media (data not shown). These results indicate that bacteriocin stability was not significantly affected by the formulation ingredients of the pork lean meat broth. The decrease in inhibitory activity could be explained by degradation or by adsorption to media constituents, but further experiments will be needed to clarify this point. Previous studies demonstrated that proteinase and peptidase activities associated with producer cells could be responsible for bacteriocin inactivation (Houlihan et al., 2004). Studies also showed that bacteriocins are less effective in food systems in which proteases are not inactivated (Vignolo et al., 1996; Castellano et al., 2004).

Another mechanism for bacteriocin inactivation has also been reported by Rose et al. (1999). The authors used MALDI-TOF/MS to demonstrate that nisin added to fresh meat and meat juices was inactivated due to a reaction with glutathione (GSH), an abundant thiol compound found in animal tissues. GSH appears to react with multiples sites on a nisin molecule in a reaction
catalyzed by glutathione S-transferase (Rose et al., 2002). In our experiments, we used filtered-sterilized pork lean meat broth and the activity of nisin Z produced by \textit{L. lactis} PD 6.9 was less than observed when the bacterium was grown in D-MRS media. These results suggest that a similar mechanism could affect the nisin Z activity of \textit{L. lactis} PD 6.9 in raw meat. Additional experiments will be needed to test this hypothesis.

The fact that antagonistic activity reached its peak when the bacteriocin producer reached stationary phase suggest that \textit{L. lactis} PD 6.9 could have an impact on the composition of the bacterial community that established during the early stages of the salami fermentation. Considering that salami fermentation and maturation can take as long as 30 days, \textit{L. lactis} PD 6.9 could become a dominant culture during the salami manufacturing process. This hypothesis is supported by previous studies in which \textit{L. lactis} PD 6.9 was isolated from salami after 6 days of fermentation and maintained its viability higher than $10^8$ CFU ml$^{-1}$ even after 22 days of processing (Maciel et al., 2003).

The prevalence and the starter culture potential of \textit{L. lactis} strain during salami fermentation was also showed by Cenci-Goga et al. (2008) and Frece et al. (2014). The authors demonstrated that \textit{L. lactis} helped improving the sensory properties of the fermented product and enhanced the inhibition of pathogens such as \textit{Listeria} spp and \textit{Staphylococcus aureus} (Cenci-Goga et al. 2008; Frece et al., 2014).

If \textit{L. monocytogenes} LMA 20 was inoculated ($10^7$ CFU ml$^{-1}$) into pork lean meat broth, growth occurred at a rate of 0.22 h$^{-1}$ and stationary phase was reached after 15 h of cultivation (Figure 2). Processed meat products can provide an excellent environment for the growth of pathogenic organisms such as \textit{L. monocytogenes} (Hereu et al., 2014; Heo et al., 2014). \textit{L. monocytogenes} a psychrotrophic and ubiquitous bacterium in meat products that is probably transferred from the environment to the food during processing (Mor-Mur and Yuste, 2010; Hereu et al., 2014). An early study performed by Martin et al. (2011) in small-scale factories producing traditional fermented sausage indicated that \textit{L. monocytogenes} could be detected in equipments (11.8% of the samples), raw materials (28.9%), and even in the final products (15.8%). Because \textit{Listeria} has shown resistance to various environmental stresses (heat, acidic pH, low water activity, low storage temperatures, etc.) commonly used in processed foods (Farber et al., 1993; Vogel et al., 2010) food industries have sought methods to control \textit{Listeria} and ensure the safety of food products.

The addition of chemical additives such as nitrite to the meat can inhibit the growth of several pathogens and ensure the safety of the processed product. However, in this study, \textit{L. monocytogenes} LMA 20 was able to grow in pork lean meat broth, even in the presence of NaCl and nitrite concentrations recommended for the fermented sausage manufacture process (Figure 2). This result could be explained in part by the higher water activity in the pork lean meat broth compared to the meat matrix of the fermented sausages, but growth of \textit{Listeria} in model sausages added with salts and chemicals has also been observed (Benkerroum et al., 2003; Albano et al., 2007; Sansawat et al., 2013). Based on higher concentrations of salt and nitrite interfere with desirable characteristics of the processed product and nitrite represent risks to the consumer's health (by affecting the ability of hemoglobin to carry oxygen or producing carcinogenic nitrosamines), alternative methods are needed to prevent the growth of \textit{Listeria} (Ananou et al., 2005; Ammor and Mayo, 2007; Freitas de Macedo et al., 2013).

One such approach could be the use of bacteriocinogenic starter cultures to control potential food-borne pathogens. Bacteriocin are ribosomally produced, extracellularly released antimicrobial peptides (post-translationally modified or not), which can have a relatively narrow spectrum of antibacterial activity (Deegan et al., 2006; Snyder et al., 2014). Bacteriocins are produced by many gram-positive and gram-negative bacteria and have been shown to inhibit the growth of several food-borne pathogens (Deegan et al., 2006; Snyder et al., 2014). Some bacteriocin-producing LAB have been isolated from fermented meat products and their ability to inhibit the growth of spoilage and pathoge-
The potential application of bacteriocins in foods can be limited by properties such as spectrum of inhibition, heat stability and solubility. In general, the following should be considered when selecting bacteriocin-producing strains for food applications: 1) the producing strains should preferably be generally recognized as safe; 2) the peptide should be heat stable and have a broad spectrum of activity against pathogens such as *L. monocytogenes* and *Clostridium botulinum*; 3) the bacteriocinogenic strain and the antimicrobial peptide should pose no associated health risks and 4) the bacteriocinogenic strains should contribute to improve safety, quality and flavour of the food products (Rodríguez et al., 2002; Snyder et al., 2014). Considering that bacteriocin-producing bacteria are frequently isolated from several food sources, it appears that many of these bacteriocinogenic strains have been safely consumed for decades. Therefore, one could argue that the reintroduction of such cultures in a food system might have negligible negative impact on the safety of the consumers (Cleveland et al., 2001).

The main concern regarding a decreased efficiency of bacteriocins as biopreservatives is related with the emergence of nisin resistant strains, particularly in *L. monocytogenes* (Begley et al., 2010; Kaur et al., 2013; 2014). Kaur et al. (2013; 2014) demonstrated that nisin-resistant *L. monocytogenes* were selected after being exposed to high bacteriocin concentrations and nisin resistant strains did not become resistant to other preservation factors, such as low pH, sodium chloride, potassium sorbate or sodium nitrite. These authors also noted that nisin-resistant *L. monocytogenes* strains were generally more sensitive to food preservatives. Therefore, bacteriocins could be used as an additional hurdle to improve food safety without being undermined by resistance (Kaur et al., 2013). However, more studies are needed to determine the distribution of bacteriocin-resistant phenotypes among microorganisms that cause food spoilage and among food borne pathogens.

**Co-culture of *L. lactis* PD 6.9 and *L. monocytogenes* in pork lean meat broth**

When *L. lactis* PD 6.9 (10⁷ CFU ml−1) and *L. monocytogenes* LMA 20 (10⁶ CFU ml−1) were co-inoculated (10:1 ratio) into pork lean meat broth, growth of *L. lactis* PD 6.9 was unaffected and the specific growth rate was similar to that observed when the culture grew alone in pork lean meat broth (Figure 3). However, no increase in *L. monocytogenes* LMA 20 cell number was observed even after 12 h of incubation. Moreover, the decrease in *L. monocytogenes* LMA 20 cell number coincided with the production of nisin Z in the cell-free supernatants of *L. lactis* PD 6.9 cultured in pork lean meat broth (Figure 1b), and viability was approximately five log units lower after 25 h of co-cultivation (Figure 3).

Preliminary plating experiments showed that *L. lactis* PD 6.9 and *L. monocytogenes* could be unambiguously distinguished and enumerated if spread onto selective media (Figure 4). Co-culture experiments indicated that growth and bacteriocin production by *L. lactis* PD 6.9 was not affected in the presence of a target organism and the inhibitory activity could be inversely correlated with *L. monocytogenes* viability (Figures 1 and 3). *L. monocytogenes* cultivated in pork lean meat broth alone approached stationary phase after approximately 15 h of incubation. However, no growth was observed during the same period if *L. lactis* PD 6.9 was also added to the medium. These results suggest that *L. lactis* PD 6.9 could outgrow *L. monocytogenes* in conditions that simulate the salami fermentation process.

Because *L. monocytogenes* is tolerant to the hurdles of the salami manufacturing process, such as low pH and high osmolarity, the decrease in viable cell number could be explained by the sensitivity of the target organism to nisin Z. This idea was further supported by co-culture experiments using *L. lactis* ATCC 19435, a non-bacteriocinogenic *L. lactis* strain. When *L. lactis* ATCC 19435 (10⁷ CFU ml−1) was co-inoculated into pork lean meat broth containing *L. monocytogenes* (10⁶ CFU ml−1), the media pH decreased, but the viable cell number of *L. monocytogenes* did not change even after 48 h of...
incubation (data not shown). Because the cell number of *L. monocytogenes* LMA 20 did not change even when the pH was as low as 4.4 or in the presence of a non-bacteriocinogenic *L. lactis* strain, the bacterial antagonism appeared to be related with the production of nisin Z by *L. lactis* PD 6.9.

*L. lactis* PD 6.9 showed inhibitory activity against 10^7 CFU ml^{-1} of *L. monocytogenes* LMA 20 even at low cell densities (Figure 5). When the cell number of *L. lactis* PD 6.9 inoculated in a co-culture varied from 10^1 to 10^7 CFU ml^{-1} (1 million fold ratio variation between *L. monocytogenes* and *L. lactis* PD 6.9), the viability of *Listeria* was reduced 10 fold with a 10^5 CFU ml^{-1} inoculum of *L. lactis* PD 6.9 (Figure 5). If the inoculum size increased to values equal to or greater than 10^6 CFU ml^{-1}, *Listeria* counts were below detection level in the co-culture after 48 hours of incubation (Figure 5). When we tested a co-culture inoculated with a *Listeria* population more plausible to be found in contaminated foods (10^6 CFU ml^{-1}), even 10^5 CFU ml^{-1} of *L. lactis* PD 6.9 were able to reduce *L. monocytogenes* LMA 20 cell counts below the detection level (1 Log_{10} CFU ml^{-1}) in pork lean meat broth (not shown results).

*L. monocytogenes* is often found in raw meat at populations lower than 10^3 cfu/g (Buchanan et al., 1987; Thevenot et al., 2006), but our results indicate that even counts as high as 10^7 CFU ml^{-1} of *L. monocytogenes* could be reduced below detection level after 48 h of incubation if *L. lactis* PD 6.9 was inoculated into pork lean meat broth at cell numbers greater than 10^4 CFU ml^{-1}. Some food industries use starter cultures to ensure the safety and quality of fermented sausages and these cultures are commonly inoculated at 10^8 CFU g^{-1}. In conditions that approached the level of contamination normally found in foods, *L. lactis* PD 6.9 could prevent the growth of *L.
monocytogenes even if co-inoculated with only 10^1 CFU ml^-1 (1000:1 ratio between L. monocytogenes and L. lactis PD 6.9). Although the effect of L. lactis PD 6.9 against other important food-borne pathogens has not yet been assessed, this bacterium could be a potentially useful starter culture in salami fermentations. Our preliminary results indicate that at least some Staphylococcus aureus strains are also inhibited by L. lactis PD 6.9. Further studies will address if L. lactis PD 6.9 also inhibits starter cultures that are used for salami fermentation or interfere with the organoleptic characteristics of product.

Although our results were obtained in a model system (liquid model) that differs in composition from traditional technological processes for sausage production, the main conditions prevailing during salami fermentation that interfere with bacterial growth were maintained. It is well known that many bacteriocins behave differently in liquid and solid matrices, but the antimicrobial activity of nisin has been demonstrated in real sausages (Hampikyan and Ugr, 2007).

These results indicate that L. monocytogenes is inhibited in conditions that prevail during salami fermentation when co-cultured with L. lactis PD 6.9. The antagonistic and competitive properties of L. lactis PD 6.9 are relevant for its application as a starter culture for fermented sausages, even though the bacteriocin activity might be reduced by components of the salami system. Additionally, because L. lactis has been generally recognized as safe and the L. lactis PD 6.9 was obtained from a food source (Italian salami), its re-introduction in fermented salami should not impose toxicological problems for consumption of the final product. Furthermore, the use of bacteriocin-producing starter cultures is especially attractive to replace chemical additives or add new hurdles that are effective inhibiting the growth of spoilage and pathogenic bacteria during food processing.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Brazil. R.A. de Paula received a doctoral fellowship from the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG).

REFERENCES


monocytogenes on RTE cooked meat products after a high pressure treatment at 400 MPa. Int. J. Food Microbiol. 186:84-94.


African Journal of Microbiology Research

Related Journals Published by Academic Journals

- African Journal of Biotechnology
- African Journal of Biochemistry Research
- Journal of Bacteriology Research
- Journal of Evolutionary Biology Research
- Journal of Yeast and Fungal Research
- Journal of Brewing and Distilling