Government of Canada / Gouvernement du Canada

Final Screening Assessment of

Micrococcus luteus strain ATCC 4698

Environment and Climate Change Canada

Health Canada

February 2018

Canada

Cat. No.: En14-313/2018E-PDF

ISBN 978-0-660-24725-0

Synopsis

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of Micrococcus luteus (M. luteus) strain ATCC 4698.

M. luteus strain ATCC 4698 is a bacterial strain that shares characteristics with other strains of the species. M. luteus belongs to the normal flora of mammalian skin and mucous membranes, and is also widespread in the environment, including soil, air, dust, water, polar ice, activated sludge, plants, fish, insects and food. It has properties that make it of potential use in bioremediation, biodegradation, wastewater treatment, drain cleaning and degreasing, growth promotion of plants and fish, skin treatment, and the production of enzymes and antibiotics.

There is no conclusive evidence in the scientific literature to suggest that M. luteus strain ATCC 4698 is likely to have adverse effects on terrestrial or aquatic plants, vertebrates or invertebrates in the environment. In a Springtail reproduction test conducted with strain ATCC 4698, no significant effects on adult survival or juvenile production were observed. There are a few reports of animal infections attributed to the species M. luteus, which are either too old to verify by using modern identification methods or were polymicrobial with 7-10 other micro-organisms involved. M. luteus was unlikely to be the primary pathogen. Moderate pathogenicity of M. luteus towards an insect pest of hazelnuts was reported under experimental conditions, which are unlikely to occur in nature.

There is no evidence from the scientific literature to suggest that M. luteus strain ATCC 4698 is likely to have adverse effects on human health. In humans, M. luteus is generally considered to be a harmless, non-pathogenic, commensal organism, and is rarely isolated as an opportunistic pathogen from damaged tissues. Early Micrococcus infections were diagnosed using methods that did not differentiate Micrococcus from coagulase-negative Staphylococcus, the more likely agent of infection. The few infections attributable to M. luteus were acquired following a medical procedure that could introduce micro-organisms from the skin into sterile body compartments, including cardiac surgery or use of central venous catheters, often in individuals with debilitating diseases, such as cancer or kidney failure. In the unlikely event of infection, M. luteus is susceptible to most antibiotics.

This assessment considers the aforementioned characteristics of M. luteus strain ATCC 4698 with respect to environmental and human health effects associated with consumer and commercial product use and in industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information

about current uses, the Government launched a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). Information submitted in response to the section 71 Notice indicates that M. luteus strain ATCC 4698 was not imported into or manufactured in Canada in 2008, except in limited quantities for academic research, teaching, and research and development activities.

Based on the information available, it is concluded that M. luteus strain ATCC 4698 does not meet the criteria under paragraph 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. It is also concluded that M. luteus strain ATCC 4698 does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Table of contents

List of tables

List of figures

Introduction

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health are required to conduct screening assessments of living organisms added to the Domestic Substances List (DSL) by virtue of section 105 of the Act, to determine whether they present or may present a risk to the environment or human health (according to criteria as set out in section 64 of CEPA).[1] M. luteus strain ATCC 4698 was added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data from Health Canada[2] and Environment Canada[3] research scientists, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain and from a mandatory CEPA section 71 notice published in the Canada Gazette, Part I, on October 3, 2009. Further details on the risk assessment methodology used are available in the Risk Assessment Framework document "Framework on the Science-Based Risk Assessment of Micro-organisms under the Canadian Environmental Protection Act, 1999" (Environment Canada and Health Canada, 2011).

In this report, data that are specific to DSL-listed M. luteus strain ATCC 4698 are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, Google Scholar, CAB abstracts), web searches and key search terms for the identification of human health and environmental hazards of the DSL strain assessed in this report. Information identified up to November 2015 was considered for inclusion in this screening assessment report.

---

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

[2] Testing conducted by Health Canada's Environmental Health Science and Research Bureau

[3] Testing conducted by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division

Decisions from domestic and international jurisdictions

Domestic

The Public Health Agency of Canada (PHAC) assigned M. luteus to Risk Group 1 (low individual risk, low community risk) for both humans and terrestrial animals (PHAC, 2011; PHAC personal communication, 2015).

M. luteus is not a concern for aquatic or terrestrial animals or plants by the Canadian Food Inspection Agency (CFIA personal communication, 2015), and is included on their list of "Organisms that do not require a Plant Protection Permit to Import" (CFIA, 2011).

International

Germany's Federal Institute for Occupational Safety and Health has placed M. luteus strain ATCC 4698 in "Risk Group 1" (DSMZ, 2015).

Micrococcus species are considered to be harmless, non-pathogenic-, commensal organisms (risk class 1, Approved List of Biological Agents 2004, Advisory Committee on Dangerous Pathogens, Health and Safety Executive of United Kingdom; HSE-UK, 2015).

No other international regulatory decisions were found regarding M. luteus[4].

[4] Government agencies and organizations searched include: the United States Environmental Protection Agency; United States Food and Drug Administration; United States Animal and Plant Health Inspection Services; United States Department of Agriculture; American Biological Safety Association; World Health Organization; United States Centers for Disease Control; Biosecurity NZ; Australian Department of Health; European Food Safety Authority; European Centre for Disease Prevention and Control; and the Invasive Species Specialist Group.

1. Hazard assessment

1.1 Characterization of Micrococcus luteus

1.1.1 Taxonomic identification and strain history

Binomial name: Micrococcus luteus

Taxonomic designation:

Kingdom:	Bacteria
Phylum:	Actinobacteria
Class:	Actinobacteria
Order:	Actinomycetales
Family:	Micrococcaceae
Genus:	Micrococcus
Species:	Micrococcus luteus (Schroeter) Cohn, 1872; emend. Wieser et al. 2002; validated Euzeby, 1997)
DSL strain:	Micrococcus luteus ATCC 4698

Synonyms, common and superseded names:

Micrococcus lysodeikticus, Sarcina citrea, Sarcina flava, Sarc ina lutea (LPSN, 2015), Bacteridium luteum (NCBI, 2015) Gaffkya tetragena, Micrococcus

Strain history:

Micrococcus luteus strain ATCC 4698 was originally deposited to the American Type Culture Collection (ATCC) as Micrococcus lysodeikticus by A. Fleming, isolated by culturing human nasal secretions for 4 days on blood agar (Fleming, 1922). It is the type strain of the species.

Other noted designations for this strain include:

AJ 1009^{T}, AS 1.2299^{T}, ATCC 15307^{T}, ATCC 4698^{T}, BCRC 11034^{T}, BUCSAV 393^{T}, CCM 169^{T}, CCRC 11034^{T}, CCT 2283^{T}, CCT 2688^{T}, CCT 2692^{T}, CCT 3024^{T}, CCTM 2979^{T}, CCTM La 2979^{T}, CCUG 5858^{T}, CDBB 72^{T}, CECT 5053^{T}, CECT 51^{T}, CECT 5863^{T}, CGMCC 1.1848^{T}, CGMCC 1.2299^{T}, CIP A270^{T}, CIPA270^{T}, CN 3475^{T}, CNCTC 6599^{T}, CNCTC M 15/65^{T}, DSM 20030^{T}, DSMZ 20030^{T}, FIRDI

1034 ^{T}, Fleming A ^{T}, GIFU 8717 ^{T}, GISK 15307 ^{T}, HAMBI 1398 ^{T}, HAMBI 1399 ^{T}, HAMBI 26 ^{T}, HUT-8101 ^{T}, IAM 1056 ^{T}, IAM 13591 ^{T}, IEGM 391 ^{T}, IEM 1056 ^{T}, IEM 65 ^{T}, IEM M 15/65 ^{T}, IEM M15 ^{T}, IFO 1056 ^{T}, IFO 3333^{T}, IMI 349015 ^{T}, IMSNU 20332 ^{T}, IMSNU 20354 ^{T}, JCM 1464 ^{T}, KACC 10488 ^{T}, KCTC 1056^{T}, KCTC 3063 ^{T}, LMD 78.1 ^{T}, LMG 4050 ^{T}, ML8 ^{T}, NBIMCC 1439 ^{T}, NBRC 3333 ^{T}, NCCB 78001 ^{T}, NCDO 947 ^{T}, NCFB 947 ^{T}, NCIB 10474 ^{T}, NCIB 9278 ^{T}, NCIM 2170 ^{T}, NCIMB 10474 ^{T}, NCIMB 9278 ^{T}, NCTC 2665 ^{T}, NRIC 1094 ^{T}, NRRL B-287 ^{T}, PCM 525 ^{T}, PCM 525 ^{T}, RIMD 1303001 ^{T}, SMG 4050 ^{T}, strain A. Fleming ^{T}, USCC 1230 ^{T}, USCC 1534 ^{T}, USCC 555 ^{T}, VKM 1314 ^{T}, VKM Ac-2230 ^{T}, VKM B-1314 ^{T}, VKM B-1314 ^{T}, VKM B-1813 ^{T}, VTT E-93442 ^{T}, WDCM 00111 ^{T} (Verslyppe et al. 2014; StrainInfo, 2014).

1.1.1.1 Phenotypic and molecular characteristics

The genus Micrococcus is the type genus of family Micrococcaceae, and was originally described by Cohn (1872). The description of the genus has been revised several times since then. The genus Staphylococcus was once included within the genus Micrococcus, and was later separated as a new genus, based on glucose utilization (Baird-Parker, 1965), and DNA guanine+cytosine (G+C) content (Rosypal et al. 1966): the genus Micrococcus retained the aerobic glucose-utilizers or non-utilizers and strains with higher G+C (66-73%), while glucose-fermenters and strains with low G+C (30-37%) were placed into the genus Staphylococcus. Four of the eight remaining subgroups of the genus Micrococcus were later moved into the genus Staphylococcus based on DNA-DNA hybridization and cell wall chemistry (Stackebrandt et al. 1995).

It is important to be able to differentiate Micrococcus from Staphylococcus in clinical microbiology, as coagulase (-) Staphylococcus has been misidentified as Micrococcus in the past (Kocur et al. 2006). In addition to differences in glucose metabolism and G+C content, Micrococcus and Staphylococcus can be differentiated using culture-based methods: Micrococcus is capable of growth on furazolidone-peptone agar, susceptible to bacitracin and the vibriostatic agent 0/129, resistant to lysostaphin, and unable to grow on a selective medium containing thiocyanate plus azide (Kocur et al. 2006). Micrococcus species also demonstrate a much slower growth and more convex colony shape than do Staphylococcus species (Kloos et al. 1974). Most strains of Staphylococcus aureus produce coagulase while M. luteus strain ATCC 4698 does not (Mortensen and Kocur, 1967). Micrococcus can also be phylogenetically differentiated from Staphylococcus based on 5S rRNA sequences (Dekio et al. 1984) in addition to 16S rRNA gene sequence analysis (Stackebrandt et al. 1995). The genus was further refined based on 16S rRNA sequence analysis, resulting in four new genera: Kocuria, Nesterenkonia, Kytococcus and Dermacoccus, being dissected from Micrococcus (Stackebrandt et al. 1995), as shown in Appendix B, Figure B-1.

Presently, the genus Micrococcus includes 10 species (LPSN, 2015):

- M. aloeverae (Prakash et al. 2014),

- M. antarcticus (Liu et al. 2000),
- M. cohnii (Rieser et al. 2013),
- M. endophyticus (Chen et al. 2009),
- M. flavus (Liu et al. 2007),
- M. lactis (Chittpurna et al. 2011),
- M. luteus (Wieser. 2002),
- M. lylae (Wieser. 2002),
- M. terreus (Zhang et al. 2010),
- M. yunnanensis (Zhao et al. 2009).

Taxonomic differentiation of Micrococcus species is more reliable by 16S rRNA gene sequence analysis, using Micrococcaceae-specific signature nucleotides at positions 293-304, 610, 598, 615-625, 1025-1036, 1026-1035, 1265-1270 and 1278 according to Escherichia coli numbering (Wieser et al., 2002). Health Canada scientists confirmed the identity of the DSL strain to be M. luteus strain ATCC 4698 using full length 16S rRNA gene sequence data by comparing to both the Microseq full gene library 2.0 (99.78% match) and the Ribosomal Database Project release 11 (0.992-0.987 match score).

A phylogenetic tree based on 16S rRNA gene sequence analyses of all 10 Micrococcus species showed that 8 of the 10 Micrococcus species, including Micrococcus luteus, cluster together, while M. lactis and M. terreus cluster more closely with Zhihengliuella and Arthrobacter species (see Figure 1-1).

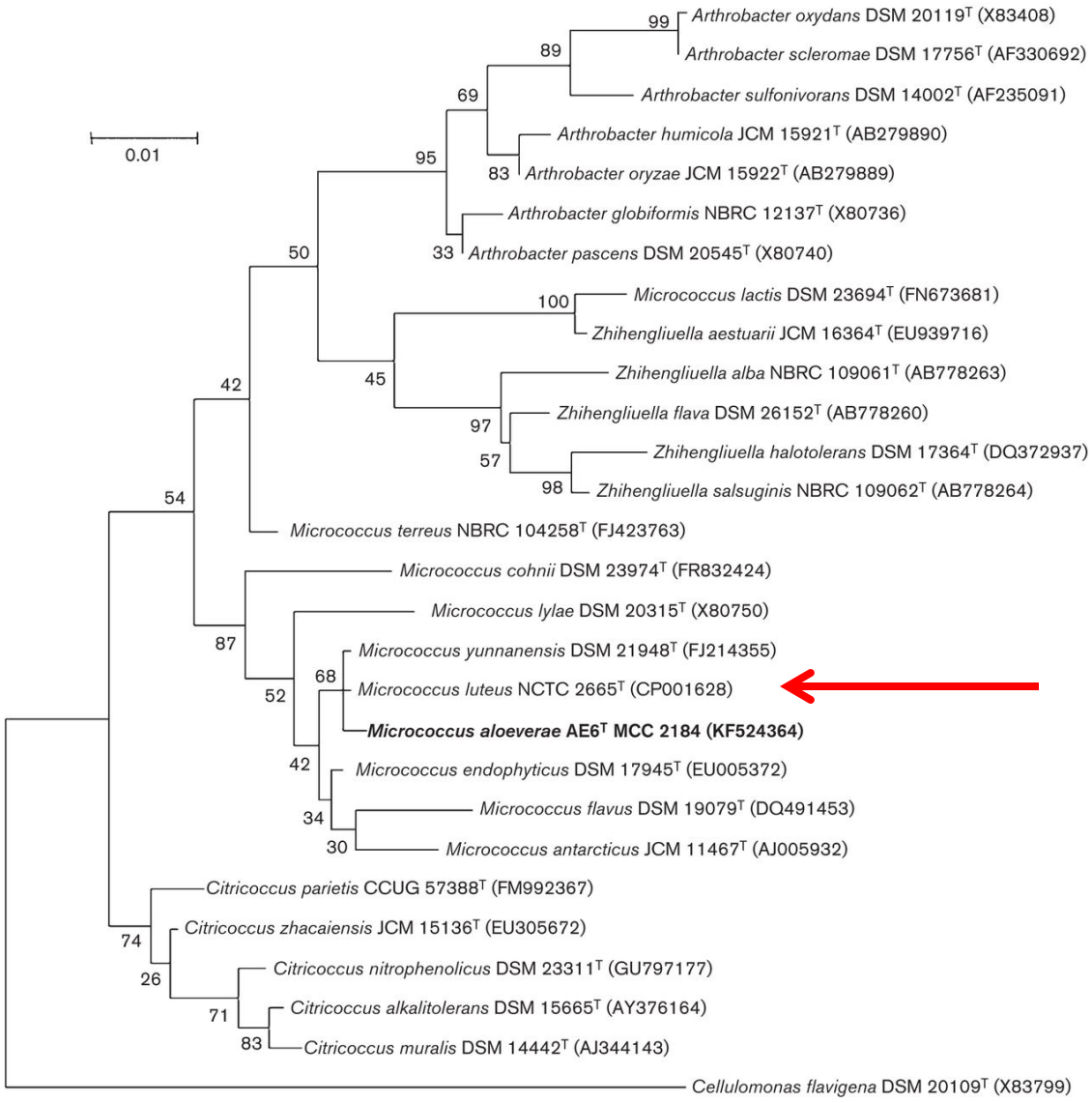

Figure 1-1: A neighbour-joining phylogenetic tree based on 16S rRNA sequences, showing the relationships among the ten Micrococcus species (Prakash et al. 2014). The arrow indicates the DSL strain.

M. luteus cells are non-motile, non-endospore forming, Gram-positive cocci, often arranged in tetrads. Creamy, yellow-pigmented colonies are typical, while cream, white or unpigmented strains have also been isolated (Kloos et al. 1974). M. luteus is chemoorganotrophic, with a strictly respiratory metabolism. It is mesophilic (optimum growth at 30-37^{o}C). The upper limits of growth for M. luteus for temperature, pH and salinity are 45°C, pH 10 and 10% NaCl, respectively. M. luteus can form dormant structures which enable the cells to survive long periods under adverse environmental conditions (Kaprelyants and Kell, 1993). M. luteus tests positive for catalase, oxidase, utilization of D-glucose, sucrose and D-mannose, and has A2 type peptidoglycan that contains L-lysine as the diagnostic amino acid, MK-8 and MK-8(H_2) are the major menaquinones (Stackebrandt et al. 1995; Wieser et al.

2002). Identification using fatty acid methyl ester analysis has been reported to yield false positives for M. luteus, requiring the identification to be confirmed by the observation of Gram positive cocci (Oka et al. 2000).

In the analysis of 10 different M. luteus strains, only utilization of D-mannose (+), L-leucine (-), 3- and 4-hydroxybenzoate (-) and yellow-pigmentation (+) tests were invariable among the strains (Wieser et al. 2002). Wieser et al. (2002) proposed 3 biovars of M. luteus based on these variable characteristics, placing the type strain M. luteus strain ATCC 4698 in biovar 1, which is more restricted in its ability to assimilate a variety of substrates than the other M. luteus biovars or the closely-related M. lylae (Table 1-1). Thus, the substrate utilization profile may be useful to differentiate ATCC 4698 from closely related strains and M. lylae species.

Table 1-1: Ability of Micrococcus luteus biovars and the closely-related species M. lylae to hydrolyse and use various substrates

Substrates	M. luteus Biovar I[a]	M. luteus Biovar II[b]	M. luteus Biovar III[c]	M. lylae[d]
D-Mannose	+	+	+	-
D-Maltose	-	+	+	+
D-Trehalose	-	+	+	+
Acetate	-	+	-	+
Propionate	+	+	-	-
DL-3-Hydroxybutryate	-	+	+	+
DL-Lactate	-	+	+	+
Oxoglutarate	-	-	+	-
Pyruvate	-	+	+	+
L-Histidine	-	+	+	+
L-Leucine	-	-	-	+
L-Phenylalanine	-	+	-	-
L-Serine	-	+	-	-
3-Hydroxybenzoate	-	-	-	+
4-Hydroxybenzoate	-	-	-	+
Phenylacetate	-	+	-	-
L-Proline pNA[e]	+	+	-	+
Casein[e]	-	+	-	-

Adapted from Wieser et al. 2002

[a] Biovar I is represented by the type strain ATCC 4698

[b] Biovar II is represented by strain D7 but also includes strains 3, 6, 7, 13C2, 38, 83,118

[c] Biovar III is represented by strain Ballarat

[d] M. lylae is represented by the type strain DSM20315

[e] Hydrolysis only

+, positive;-, negative.

In addition to its more restricted substrate assimilation profile, M. luteus can be differentiated from M. lylae on the basis of peptidoglycan, menaquinone and fatty acid composition. M. luteus contains the L-Lys peptide subunit in its cell wall peptidoglycan while M. lylae has the L-Lys-D-Asp subunit type. The menaquinone composition of M. luteus is mainly MK-8 with some MK-8(H_2) and MK-7, whereas that of M. lylae is mainly MK-8(H_2), with small amounts of MK-7(H_2) and MK-9(H_2) (Stackebrandt et al. 1995). Approximately 4% of the fatty acids of M. luteus strain ATCC 4698 are $C_{16:1}\omega7c$, whereas this type is undetected in M. lylae. Conversely, 3.7% of the fatty acids of M. lylae are of i-$C_{17:0}$ and ai-$C_{17:0}$, of which only trace amounts are found in M. luteus type strain (Wieser et al. 2002).

M. luteus strain ATCC 4698 is phylogenetically related to Kocuria rhizophila, which was formerly considered a M. luteus strain (ATCC 934) based on 5S rRNA sequences (Dekio et al. 1984). This relatedness was also confirmed by genomic sequence analysis (Young et al. 2010). However, physiological differences between ATCC 9341 and ATCC 4698 ultimately resulted in the classification of the former as K. rhizophila (Tang and Gillevet, 2003; see Appendix, Table A-1).

1.1.2 Biological and ecological properties

1.1.2.1 Natural occurrence

M. luteus strains are ubiquitous in the environment and considered to be part of the normal flora of the mammalian skin. M. luteus strains have been isolated from diverse habitats:

Mammalian skin

- human skin of the head, legs and arms (Kloos et al. 1974; Kloos and Musselwhite, 1975),
- skin of a variety of mammals, including squirrels, rats, raccoons, opossums, horses, swine, cattle, dogs, and various primates (Kloos et al. 1976).

In association with other animals and plants

- intestine of nile tilapia (Oreochromis niloticus) (Abd El-Rhman et al. 2009),
- mucus of the red sea coral (Fungia scotia) (Lampert et al. 2006).
- living sponge (Bultel-Poncé et al. 1998),
- summer chafer, an insect pest of hazelnuts (Amphimallon solstitiale L) (Sezen et al. 2005),
- grapevine (Altalhi 2009).

Soil and Water

- drinking water (Rusin et al. 1997),
- a biofilm in a freshwater tank (Rickard et al. 2003).

- sea surface microlayers of polluted waters (Agogué et al. 2005)
- soil (Sims et al. 1986; Biskupiak et al. 1988),

Wastewater and contaminated sites

- activated sludge plant (Wieser et al. 2002),
- effluent of the textile industry (Bari and Bhardwaj, 2014),
- nitrobenzene-contaminated activated sludge (Zheng et al. 2009)
- oligotrophic lake containing pesticides (López et al. 2005).

Extreme environments

- polar ice (Antony et al. 2012),
- microbial mats of Antarctic lakes (Van Trappen et al. 2002),
- 120 million year old amber (Greenblatt et al. 2004).
- alkaline groundwater (pH 11.4) (Tiago et al. 2004),

M. luteus is also frequently isolated from sites that have likely become contaminated with M. luteus through contact with mammalian skin, either through direct human contact or shedding of skin:

Indoor air

- in a museum (Wieser et al. 2002),
- in a suburban elementary school (Kooken et al. 2012).

Surface dust

- a medieval wall painting in a chapel (Wieser et al. 2002)
- in space in the MIR and the ISS stations (Gu, 2007),

Medical settings

- stethoscopes (Marinella et al. 1997).
- otolaryngology equipment (Powell et al. 2003),
- orthodontic buccal tubes (Purmal et al. 2010),
- doctors' cell phones (Tambekar et al. 2008), 0
- rinse water of washer disinfectors (Martin et al. 2008),
- a contaminant in 4 lots of a recalled drug, cefazolin (US-FDA, 2006)

Food (as a contaminant)

- cheese (Addis et al. 2001; Prado et al. 2001),
- a dry-fermented sausage in Spain (García Fontán et al. 2007),
- beer (Pittet et al. 2010)

1.1.2.2 Survival, persistence and dispersal in the environment

Although M. luteus is normally copiotrophic (Kaprelyants and Kell, 1993), it can survive oligotrophic conditions (Dib et al. 2013). M. luteus can form non-endospore dormant structures (Kaprelyants and Kell, 1993; Kaprelyants et al. 1993, Mukamolova et al. 1995, 2002, 2006; Votyakova et al. 1994), which enable the cells to survive long periods under adverse environmental conditions, such as starvation and dryness (Kaprelyants et al. 1993). The adaptability of M. luteus to extreme environments has been attributed to these dormant structures (Dib et al. 2008; Ordonez et al. 2009). For example, live M. luteus was isolated from 120 million year old amber (Greenblatt et al. 2004). In another example, populations of M. luteus strain ATCC 4698 declined by < 2 $\log_{10}$ after 25 days post inoculation onto dry cotton, indicating its resistance to dryness (Hirai, 1991). This viable but non-culturable state ends when favourable conditions trigger a “revival factor or resuscitation promoting factor” coded by the gene rpf (Mukamolova et al. 2002; Greenblatt et al. 2004). In contrast to most Actinobacteria, M. luteus has only one copy of the rpf gene (Young et al. 2010). However, the significance of this is not well-understood.

M. luteus cells became undetectable within 3 weeks of inoculation into soil at 2.5 x 10^7 cells/g (dry weight) soil. The disappearance of M. luteus was attributed to the presence of bacterial predators (Casida, 1980a), including a filamentous organism similar to Streptoverticillium species and an unidentified Gram-negative rod shaped bacterium (Casida, 1980b). Later, Myxococcus xanthus was also shown to be one of the predators of M. luteus (Hillesland et al. 2007). Cold (4°C), dryness (2.5% humidity) and starvation increased the survival of M. luteus in soil (Casida, 1980a), possibly by creating unfavorable growth conditions, and thereby inducing dormacy (Dib et al. 2013).

M. luteus strain ATCC 4698 is susceptible to antibacterial compounds produced by other micro-organisms, which could make it a poor competitor outside of its niche environment. For example, it is susceptible to antibacterial activities of some strains of Aeromonas caviae, Aeromonas hydrophila, Aeromonas jandaei, Aeromonas sobria, Bacillus species, Enterobacteriaceae, Bacterioides type A, Bacterioidaceae and Clostridium species isolated from the intestines of various freshwater fish including Common Carp, Rainbow Trout, and Tilapia in Japan (Sugita et al. 1996); some psychrotrophic bacterial strains isolated from Antarctica (Lo Giudice et al. 2007), S. aureus 4185 (Ceotto et al. 2010), and Lactobacillus rhamnosus (Dimitrijević et al. 2009), and the dermatophyte, Trichophyton mentagrophytes (Bibel and Smiljanic, 1979). M. luteus can form biofilms in both pure and mixed cultures. However, in some mixed cultures, M. luteus is quickly outcompeted and becomes undetectable because of antagonism by other micro-organisms, such as Pseudomonas aeruginosa and S. aureus (Malic et al. 2011).

1.1.2.3 Growth parameters

M. luteus is a mesophilic aerobe (DSMZ, 2015), which thrives at temperatures from approximately 15°C to 40°C. Recommended growth conditions for M. luteus strain ATCC 4698 are 30°C on Nutrient Agar/Broth (DSMZ, 2015; ATCC, 2015) although it also grows well at 37°C (Kocur et al. 2006). Under favourable conditions (e.g. nutrient agar supplemented with 0.5% w/v L-lactate and 0.05% yeast extract) the doubling time is approximately 4 hours (Kaprelyants and Kell, 1993).

1.1.2.4 Genomic features and horizontal gene transfer

M. luteus strain ATCC 4698 has one of the smallest genomes of any free-living actinobacterium sequenced to date, which consists of a single circular chromosome spanning 2,501,097 bp with 73% G+C content and encoding 2,403 predicted proteins, (Young et al. 2010). Some of the main features of M. luteus strain ATCC 4698 are:

- 73 insertion sequence (IS) elements, most showing homology with corresponding sequences in other Actinobacteria;
- 4 sigma factors and 14 response regulators, possibly reflecting a high degree of adaptation to the preferred habitat of mammalian skin;
- very few genes involved in secondary metabolism;
- a 3-gene cluster associated with long-chain alkene biosynthesis
- only one gene for a resuscitation-promoting factor (Rpf);
- lacks the wblC gene associated with antibiotic resistance;
- a reduced set of penicillin-binding proteins possibly reflective of its sensitivity to β-lactam antibiotics; and
- no gene for glucokinase.

A recent review of the extrachromosomal genetic elements in Micrococcus species describes the presence of a number of circular and linear plasmids and bacteriophages and transposable elements which play a role in horizontal gene transfer (Dib et al. 2013). Most plasmids are associated with degradation of chemicals in the environment and resistance to chemicals, heavy metals and antibiotics. These plasmids and associated phenotypes of Micrococcus species are listed in Appendix Table B-1.

1.1.2.5 Resistance/sensitivity to antibiotics and disinfecting agents

M. luteus is sensitive to most antibiotics, including penicillin, gentamicin, clindamycin, and vancomycin (Bannerman and Peacock, 2007). However, resistance to penicillin G, tetracycline, clindamycin, nitrofurantoin, erythromycin and lincomycin have been observed in some strains (Lampert et al. 2006; Liebl et al. 2002; Magee et al. 1990). Seven of nine M. luteus strains isolated from biofilms formed in the endotracheal tubes of 9/20 patients in a university hospital in Belgium were resistant to oxacillin (Vandecandelaere et al. 2013).

Antibiotic susceptibility testing of M. luteus strain ATCC 4698 was also performed by Health Canada Scientists (Table 1-2).

Table 1-2: Minimum inhibitory concentrations (MICs)[a] for antibiotics against M. luteus strain ATCC 4698

Antibiotic	MIC (mean ± SD)	MIC for Clinical Breakpoint (mg/L)
Amoxicillin (S)	0.035 ± 0.023	S≤2 R>8
Cefotaxime (S)	0.12 ± 0	S≤1 R>2
Ciprofloxacin (R)	1 ± 0	S≤0.5 R>1
Clindamycin (IE)	0.05 ± 0.03	IE
Imipenem (S)	0.009 ± 0.006	S≤2 R>8
Levofloxacin (R)	2 ± 0	S≤1 R>2
Linezolid (S)	0.67 ± 0.29	S≤2 R>2
Meropenem (S)	0.015 ± 0	S≤2 R>8
Oxacillin (IE)	2.0 ± 1.7	IE
Tetracycline (S)	1.17 ± 0.78	S≤2 R>2
Tigecycline (R)	0.83 ± 0.29	S≤0.25 R>0.5
Vancomycin (IE)	0.38 ± 0.14	IE

There is insufficient evidence (IE) about MIC values and clinical relevance unless otherwise stated as (R) for resistance and (S) for sensitivity according to EUCAST, 2015.

[a] Tests performed by Health Canada's Environmental Health Sciences and Research Bureau (Antibiotic Strips-Oxoid®, incubated 37^0C, 24h, TSB agar)

M. luteus biofilms formed on stainless steel surfaces are more sensitive to disinfecting agents hydrogen peroxide and peroxyacetic acid than the biofilms formed by other bacteria, such as Listeria innocua, Pseudomonas putida and Staphylococcus hominis (Królasik et al. 2010). M. luteus strain ATCC 4698 is susceptible to 0.03% chlorine dioxide, 7.5% hydrogen peroxide or 2.25% peracetic acid, causing >5 $\log_{10}$ reduction in cell viability after 30 seconds exposure (Martin et al. 2008). M. luteus strain ATCC 4698 is also sensitive to: Schiff bases and their derivatives (Panneerselvam et al. 2005 and 2009; Shingade and Bari, 2013), some coumarin triazole derivatives with MICs as low as 1 µg/mL (Shi and Zhou, 2011), and human lactoferrin (de Lillo et al. 1997). An unnamed strain of M. luteus was reported to be sensitive to silver nanoparticles (MIC= 8.8 µg/µl) much more than silver nitrate as determined by its larger zone of inhibition (19 mm vs 9 mm) on agar plates (Balashanmugam and Kalaichelvan, 2015).

M. luteus strain ATCC 4698 possesses UV resistance conferred by a UvrABC-like nucleotide excision repair mechanism (Piersen et al. 1995, Zherebtsov and Tomilin, 1975). In spite of this, M. luteus strain ATCC 4698 is sensitive to germicidal UV radiation (200-260 nm) under which it is inactivated more efficiently at low humidity (20-25%) than higher humidity (90-95%) (Gorsuch et al. 1998). M. luteus is sensitive to high pressure carbon dioxide, and treatment with 50 bar carbon dioxide at 65°C completely inactivated M. luteus strain ATCC 4698 on hospital-type fabric (50% cotton and 50% polyester) (Cinquemani et al. 2007). The effect was dependent on

the presence of as little as <1% v/v water, suggesting that the mechanism of this effect may be the formation of carbonic acid within the cell.

1.1.2.6 Pathogenic and toxigenic characteristics

A soil isolate, M. luteus ATCC 53598, produced the novel sulfur-containing antibiotic neoberninamycin, which was inactive against Gram-negative aerobes but active against both aerobic and anaerobic Gram-positive bacteria (Biskupiak et al. 1988). There is no evidence in the literature of strain ATCC 4698 producing this antibiotic.

Like other Gram-positive bacteria, the cell wall of M. luteus contains teichuronic acid, peptidoglycan and lipoglycans, which, like many bacterial cell wall components, are potent immunostimulatory molecules. Purified teichuronic acid and lipoglycan from M. luteus were shown to induce cytokines, such as tumour necrosis factor-α in mice (Monodane et al. 2001) and interleukin-6 in a human macrophage cell line (Blanc et al. 2013), respectively. Peptidoglycan fragments from M. luteus induce lysozyme production in larvae of the tobacco hornworm (Manduca sexta) (Kanost et al. 1988), and induce antibacterial protein synthesis in silkworm (Bombyx mori) larvae (Iketani et al. 1999). Lysozyme can neutralize the proinflammatory effects of peptidoglycan; lysozyme-deficient mice experience extensive tissue injury when experimentally injected with M. luteus strain ATCC 4698 and this effect is the same whether M. luteus cells are live or killed, suggesting that inactivation of peptidoglycan, not a bacteriocidal effect of lysozyme, is protective (Ganz et al. 2003).

Extensive literature searches found no other virulence factors or toxins in M. luteus.

1.1.3 Effects

1.1.3.1 Environment

There are no reports in the publicly available literature directly implicating M. luteus strain ATCC 4698 in adverse effects on aquatic or terrestrial vertebrates and invertebrates or plants.

Aquatic vertebrates

M. luteus strain ATCC 4698 is sensitive to the antibacterial effects of several microbial species of the intestinal flora of fish, including rainbow trout (Sugita et al. 1996). However, M. luteus was also isolated together with other bacteria from an outbreak of rainbow trout fry syndrome in farmed British rainbow trout (Austin and Stoble, 1992). The fish experimentally injected with the same M. luteus strain showed 54% mortality (Austin and Stoble, 1992).

A strain of M. luteus was reported to have caused chronic and sporadic infections in farmed rainbow trout over the course of four years (1993-1996), resulting in lesions on the skin and caudal fin as well as lesions on muscle, liver and spleen (Aydin et al.

2005). Infections were not observed again in the following five years. However, the strain was identified using a limited number of biochemical tests and was recorded as being motile, which is not typically a characteristic of M. luteus, so it may have been misidentified. Moreover, the organism lacks genes for synthesis and assembly of flagella and this fact alone serves to cast very serious doubt on identification of the causative organism as M. luteus.

In contrast to the above report, a native strain of M. luteus isolated from Nile tilapia (O. niloticus) and used as a probiotic for the same fish showed some in vitro antibacterial activity against fish pathogen A. hydrophila and reduced mortality caused by this organism in vivo (Abd El-Rhman et al. 2009).

Terrestrial vertebrates

M. luteus was identified as one of eight bacterial species (Bacillus cereus, Corynebacterium pyogenes, E. coli, M. luteus, Pasteurella haemolytica, Pasteurella multocida, P. aeruginosa and S. aureus) isolated in mastitic milk samples from Camels (Fazlani et al. 2008). M. luteus was also identified as one of eleven bacterial species (Streptococcus pyogenes, Strptococcus uberis, S. aureus, Streptococcus intermedius, Corynebacterium diphtheria, C. pyogenes, E. coli, Proteus vulgaris, P. aeruginosa, M. luteus and Stomatococcus mucilaginosus) isolated from surgical and non-surgical wounds of buffaloes, cattle, sheep and goats (Khan and Rind, 2001). Since M. luteus is part of the natural flora of these animals, and it was isolated with other bacteria that are more likely candidates for causing these infections, it is unlikely that M. luteus was the primary pathogen in the above two cases.

Aquatic invertebrates

M. luteus appears to be a normal inhabitant of aquatic ecosystems, yet no adverse effects of M. luteus on aquatic invertebrates have been reported in the literature. M. luteus is susceptible to antibacterial compounds in fractions of coral (Leptogorgia virgulata) tissue (Shapo et al. 2007), suggesting that corals are protected from infection with M. luteus.

Terrestrial invertebrates

A strain identified as M. luteus based on morphological, physiological and biochemical tests showed moderate biocontrol potential against summer chafer (A. solstitiale L), producing 30% mortality in larvae when included in the diet in high quantity (100 µg/mL).This was compared to 90% mortality from an isolate identified as B. cereus and 72% from an isolate identified as Bacillus thuringiensis (Sezen et al. 2005). Environment and Climate Change Canada Research scientists performed a springtail reproduction test using M. luteus strain ATCC 4698 on the common soil invertebrate Folsomia candida , according to standardized test method EPS 1/RM/44/ (Environment Canada, 2014). No significant adverse effects of M. luteus

strain ATCC 4698 on adult survival or juvenile production were observed (unpublished data by Environment Canada).

M. luteus is susceptible to antibiotic activity of the hemolymph of four millipede and centipede species: Chicobolus species, Rhapidostreptus virgator, Lithobius forficatus and Scolopendra cingulata, suggesting that these arthropods are protected from infection with M. luteus (Xylander and Nevermann, 1990).

Plants

Recently, one strain identified as M. luteus strain SUBF006, was demonstrated using Koch's postulates to be a phytopathogen for the leaves of Mango plant (Mangifera indica L. vr. Nylon) in India, and genes involved in its virulence were identified by whole genome sequencing. SUBF006, however, shows little resemblance to the ATCC 4698 since it has a 50% bigger genome size (3.86 MB) than the DSL strain (2.5 MB), and the 439 nt partial 16S rRNA gene sequence of SUBG006 shares only 94% identity with the 1418 nt 16S rRNA gene sequence of ATCC 4698, and matches over 100 other strains of Micrococcus. (Rakhashiya et al. 2015).

No other adverse effects of M. luteus on terrestrial or aquatic plants were found in the literature. One of the desiccation-tolerant strains of M. luteus isolated from the rhizosphere of an unnamed desert plant showed a plant growth promoting effect. Inoculation of corn (Zea mays) with this strain increased the number of leaves, shoot and root length, and increased dry weight per gram of fresh weight by 54% (Raza and Faisal, 2013).

1.1.3.2 Human health

There are no reports in the publicly available literature clearly implicating M. luteus strain ATCC 4698 in adverse effects on humans.

Generally, M. luteus is considered to be a harmless, non-pathogenic, commensal organism that is rarely isolated as an opportunistic pathogen. Older case reports of infection were diagnosed using methods that did not differentiate between coagulase-negative Staphylococcus and Micrococcus. Many older reports of Micrococcus infections were later attributed to various Staphylococcus species (reviewed by Kocur et al. 2006). The vast majority of 212 micrococcal urogenital tract isolates (identified by conventional methods) were re-identified as Staphylococcus after re-testing with API Staph test, and only 5 were not verified by API Staph (Baldellon and Megraud 1985). Furthermore, Seifert et al. (1995) reviewed 16 cases of endocarditis involving prosthetic heart valves and cardiac surgery that were originally attributed to Micrococcus species, and found that microbiological information provided in these publications was not sufficient to differentiate Micrococcus from coagulase-negative Staphylococcus species. As a result, only a few of the reported cases would now be accepted as Micrococcus

species. Kocur et al. (2006) suggested that data from the mid-1990s onwards should be considered for assessing the pathogenicity of M. luteus.

The following reported cases have been attributed to M. luteus in the literature from 1995 to present:

- one case of prosthetic valve endocarditis in a 71 year old man (Seifert et al. 1995);
- one case of central venous catheter-related bacteremia in a 48 year old female, kidney patient after hemodialysis (Peces et al. 1997);
- one case of central venous catheter-related bacteremia in a 14 year old male cancer patient who had been treated earlier for a Klebsiella pneumoniae infection, and later died during treatment (Shanks et al. 2001);
- 28 cases of bacteremia after medication with central venous catheters were identified in a seven-year survey from 2002 to 2008 (Hirata et al. 2009);
- one case of transient bacteremia in a teenager after removal of an orthodontic appliance (Gürel et al. 2009);
- one case of bacteremia in a 57 year old male with aseptic liver abscesses (Andreopoulos et al. 2000);
- one case of native valve endocarditis in a 74 year old female, cancer patient who also had knee replacement surgery (Miltiadous and Elisaf 2011).

There are also a few reported infections attributed to Micrococcus, in which the species was not identified. These have been included in this report as M. luteus could have been the agent isolated:

- three cases of peritionitis in peritoneal dialysis patients, one of which was polymicrobial with Pseudomonas oryzihabitans, coagulase-negative Staphylococcus species and non-fermentative Gram-negative bacteria (Kao et al. 2014);
- 90 cases of bacteremia in cancer patients identified in a ten-year survey from 1997 to 2006 (Ramos et al. 2009);
- fatal pulmonary hemorrhage in two children with acute lymphoblastic leukemia (Payne et al. 2003);
- three cases of folliculitis in HIV-1 patients (Smith et al. 1999);
- six cases of bacteremia: two cases related to central venous catheters in leukemia patients; three cases associated with peritoneal dialysis and one case related to a ventricular-peritoneal shunt (Magee et al. 1990).

In most of the above-noted cases, M. luteus was the sole organism isolated. Infections were treated successfully with antibiotics in most cases, or deaths were due to the underlying medical conditions of the patients. Since Micrococcus species are part of the healthy skin flora, their role in disease is thought to be limited, but the organism appears to be capable of attacking damaged tissues (Seifert et al. 1995).

1.2 Hazard severity

1.2.1 Environment

The environmental hazard potential of M. luteus strain ATCC 4698 is assessed to be low because despite of the widespread occurrence of M. luteus in the environment, the evidence from the scientific literature suggests that M. luteus does not adversely affect aquatic or terrestrial vertebrates, invertebrates or plants at the population level in the environment.

There are no reports in the scientific literature directly implicating M. luteus strain ATCC 4698 in adverse effects on the environment.

No adverse effects of M. luteus have been observed in aquatic or terrestrial invertebrates. While a moderate biocontrol effect of M. luteus towards an insect pest of Hazelnuts was observed under experimental conditions, a Springtail reproduction test using M. luteus strain ATCC 4698 did not elicit adverse effects on adult survival or juvenile production. M. luteus is susceptible to the antimicrobial defenses of invertebrate species including Corals, millipedes and centipedes. No other adverse effects in aquatic or terrestrial plants have been attributed to M. luteus. Instead, M. luteus shows promise as a plant growth promoting bacterium of corn.

1.2.2 Human health

The human hazard potential of M. luteus strain ATCC 4698 is assessed to be low. There are no reports in the scientific literature directly implicating M. luteus strain ATCC 4698 in adverse effects on human health. Most M. luteus infections, and particularly urinary tract infections, reported before the mid-1990s were later re-identified as Staphylococcus. A small number of cases of human infection with M. luteus, which is universally present on human skin, have been reported since 1995. Almost all followed a medical procedure that could introduce micro-organisms from the skin into sterile body compartments, often in individuals with debilitating diseases, such as cancer or kidney failure. In the unlikely event of infection with the DSL strain, M. luteus is sensitive to many antibiotics.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[5].

[5] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which

2. Exposure assessment

2.1 Sources of exposure

This assessment considers exposure to M. luteus strain ATCC 4698 resulting from its deliberate addition to consumer or commercial products and its use in industrial processes in Canada.

M. luteus strain ATCC 4698 was nominated to the DSL for use in combination with enzymes and other micro-organisms in wastewater drains, sewers, grease traps, septic systems and wastewater treatment facilities.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal regulatory and non-regulatory programs, indicated that that 10,000 to 100,000 kg of products potentially containing M. luteus strain ATCC 4698 (formulation and concentration unknown) were imported into or manufactured in Canada in 2006-2007 for use in consumer and commercial products.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). The section 71 notice applied to any persons who, during the 2008 calendar year, manufactured or imported M. luteus strain ATCC 4698, whether alone, in a mixture or in a product. The results indicated that M. luteus strain ATCC 4698 was not imported or manufactured in Canada in 2008, except for limited quantities used for academic research, teaching, and research and development activities.

The 2007 and 2009 surveys differed significantly in target and scope. In this assessment, results from the 2009 survey were used to estimate exposure from current uses because it requested information on uses of the specific strain of the micro-organism that is listed on the DSL, whereas the 2007 survey asked about uses of the products that had been associated with the micro-organism at the time it was nominated to the DSL. Because product formulations may have changed, information from the 2009 survey may more accurately represent current uses. Uses reported in the 2007 voluntary survey were also considered in the assessment of potential uses.

---

is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

Although, no large quantities were reported for ATCC 4698 during the mandatory survey, it is available for purchase from the ATCC. It could be an attractive choice for commercialization given it is on the DSL and so can be used in Canada without prior notification. A search of the public domain (MSDS, literature and patents) revealed the following consumer, commercial and industrial applications for other strains of M. luteus. These represent possible uses of the DSL strain, as strain ATCC 4698 is likely to share the characteristics (modes of action) with other commercialized M. luteus strains:

- biodegradation and bioremediation of toxic pollutants (reviewed in Dib et al. 2013):
    - petroleum (Austin et al. 1977),
    - pyridines (Sims et al. 1986; Sims and O'Loughlin, 1992),
    - chlorinated biphenyls (Bevinakatti and Ninnekar, 1993),
    - nitrobenzene (Zheng et al. 2009),
    - dyes, such as Methyl Red, Methyl Orange, Crystal Violet, Erichrome Black and Malachite Green (Bari and Bhardwaj, 2014), phthalate esters (Eaton and Ribbons, 1982),
    - polyacrylonitrile polymers (Fischer-Colbrie et al. 2007),
    - naphthalene (Zhuang et al. 2003),
    - pesticides (López et al. 2005).

- accumulation of heavy metals from the environment:
    - gold (Levchenko et al. 2002),
    - strontium (Faison et al. 1990),
    - copper and lead (Puyen et al. 2012)

- as a production organism for:
    - long-chain hydrocarbons as biofuels, lubricants (Beller et al. 2010; Young et al. 2010),
    - the antibiotic neoberninamycin (Biskupiak et al. 1988),
    - glutaminases (Chantawannakul et al. 2003),
    - esterases (Akita et al. 2001),
    - proteases (Clark et al. 2000; Manikandan et al. 2011),
    - ribavirin (Fujishima and Yamamoto, 1986),
    - L-aspartyl-L-phenyl-alanine esters (Yokozeki et al. 1987),
    - 2,6-diaminopurine-2'-deoxyriboside and 2'-deoxyguanosine (Yokozeki et al. 2001),

- growth promotion of plants (Raza and Faisal, 2013),
- growth promotion of fish (Abd El-Rhman et al. 2009),
- a probiotic nutritional preparation (Van Hoey-De-Boer and Hageman,1999),
- part of a dairy product (Vermin and Spinnler, 2004),
- a skin treatment composition for odour control and biocontrol of bacterial skin disorders (Tagg et al. 2006a, 2006b, 2012),
- an ingredient in sunscreen (SINTEF, 2013),

- a test organism for lysozyme preparations (Herrmann and Klein, 1999).

These represent possible uses of the DSL strain, as strain ATCC 4698 is likely to share the characteristics (modes of action) with other commercialized M. luteus strains.

2.2 Exposure characterization

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the overall environmental exposure estimation for M. luteus strain ATCC 4698 is low. Nevertheless, given the range and scale of known and potential applications of the species M. luteus described in Section 2.1, there is potential for an increase in environmental exposure to M. luteus strain ATCC 4698, and exposure scenarios arising from these uses have therefore been considered.

M. luteus strains can metabolize a wide range of toxic chemicals which makes them of potential use for biodegradation and bioremediation. They can also bind dissolved metals from aqueous solutions, with applications both in bioremediation and recovery of precious metals. Other potential uses include growth promotion of terrestrial plants and fish. Should these potential uses of M. luteus be realized for strain ATCC 4698 in Canada, it is likely to be introduced into both terrestrial and aquatic ecosystems. Exposure to M. luteus strain ATCC 4698 could also occur via wastewater or effluent from facilities using M. luteus strain ATCC 4698 as a production organism for enzymes and other biomolecules; however, adherence to good manufacturing practices would be expected to limit releases of the micro-organism.

The extent of exposure to M. luteus strain ATCC 4698 will depend on the quantity released, and on its survival, persistence and dispersal in the receiving environment. M. luteus does not persist in soil under natural conditions (Casida, 1980a). Nevertheless, under dry, cold or nutrient-starved conditions, some cells may persist as dormant structures, remaining viable until suitable conditions for growth arise (Dib et al. 2008). As a normal inhabitant of aquatic ecosystems, M. luteus introduced into aquatic environments could be expected to persist; however, M. luteus is susceptible to predation and anti-bacterial activities of other organisms, which is expected to limit its ability to maintain elevated populations in both terrestrial and aquatic eco-systems, and the effect of microbiostasis (Leung et al. 1995; van Veen et al. 1997) is expected to eventually restore introduced populations to background levels.

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to responses to the section 71 notice, the overall human exposure estimation for M. luteus strain ATCC 4698 is low. Nevertheless, given the range and scale of

known and potential applications of the species M. luteus listed in Section 2.1, there is potential for increase in human exposure to M. luteus strain ATCC 4698.

Should potential uses identified in section 2.1 be realized in Canada, probiotics, pharmaceuticals or cosmetics uses would provide the greatest sources of direct human exposure for strain ATCC 4698. Since M. luteus is part of the normal flora of human skin and mucosa and ATCC 4698 was originally isolated from human mucosa, exposure to the organism will cause a temporary clonal increase of M. luteus strain ATCC 4698.

Direct exposure of the general population would not be expected in the event M. luteus strain ATCC 4698 is used in Canada for bioremediation/biodegradation of toxic compounds, metal binding or as a production organism. Indirect exposure to M. luteus strain ATCC 4698 could occur in the vicinity of the treated sites, but is not expected to be greater than direct exposure during the product application. Indirect exposure to M. luteus strain ATCC 4698 in soils, subsequent to its use as a plant growth promotant or in bodies of water subsequent to its use as a fish probiotic could also occur.

In the event that the organism enters municipal drinking water treatment systems through release from potential uses, the water treatment process, which includes coagulation, flocculation, ozonation, filtration and chlorination, is expected to effectively eliminate these micro-organisms from drinking water.

3. Risk characterization

In this assessment, risk is characterized according to a paradigm whereby a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard, and on what is known about exposure from current uses.

Hazard has been estimated for M. luteus strain ATCC 4698 to be low for the environment and for human health. Environmental and human exposure to M. luteus strain ATCC 4698 is not currently expected (low exposure), so the risk associated with current uses is estimated to be low for both the environment and human health.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses).

M. luteus strains have useful properties that could result in future increases in environmental and human exposure to products containing this strain, if these foreseeable uses are realized in Canada. However, the risk from foreseeable future exposures is expected to remain low.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that M. luteus strain ATCC 4698 is not entering the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect in the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that this substance does not meet the criteria as set out in section 64 of the CEPA 1999.

5. References

Abd El-Rhman AM, Khattab YAE, Shalaby AME. 2009. Micrococcus luteus and Pseudomonas species as probiotics for promoting the growth performance and health of nile tilapia, Oreochromis niloticus. Fish and Shellfish Immunology 27(2):175-80.

Addis E, Fleet GH, Cox JM, Kolak D, Leung T. 2001. The growth, properties and interactions of yeasts and bacteria associated with the maturation of camembert and blue-veined cheeses. International Journal of Food Microbiology 69(1–2):25-36.

Agogué H, Casamayor EO, Bourrain M, Obernosterer I, Joux F, Herndl GJ, Lebaron P. 2005. A survey on bacteria inhabiting the sea surface microlayer of coastal ecosystems. FEMS Microbiology Ecology 54(2):269-80.

Akita K, Naitou C, Maruyama K. 2001. Purification and characterization of an esterase from Micrococcus sp. YGJ1 hydrolyzing phthalate esters. Bioscience, Biotechnology, and Biochemistry 65(7):1680-3.

Altalhi AD. 2009. Plasmids profiles, antibiotic and heavy metal resistance incidence of endophytic bacteria isolated from grapevine (vitis vinifera L.). The African Journal of Biotechnology 8(21):5873-82.

Andreopoulos T, Papanikolaou G, Politou M, Konstantopoulos K, Stefanou J, Loukopoulos D. 2000. Micrococcus luteus: a putative cause of hepatic abscess? Panminerva medica 42(3):231-2.

Antony R, Krishnan KP, Laluraj CM, Thamban M, Dhakephalkar PK, Engineer AS, Shivaji S. 2012. Diversity and physiology of culturable bacteria associated with a coastal antarctic ice core. Microbiological Research 167(6):372-80.

ATCC. 2015. *Micrococcus luteus* (Schroeter) Cohn (ATCC® 4698™) http://www.atcc.org/products/all/4698.aspx#culturemethod

Austin B, Calomiris JJ, Walker JD, Colwell RR. 1977. Numerical taxonomy and ecology of petroleum-degrading bacteria. Applied and Environmental Microbiology 34(1):60-8.

Austin, B. and M. Stobie, 1992. Recovery of Micrococcus luteus and presumptive Planococcus from moribund fish during outbreaks of rainbow trout (Oncorhynchus mykiss Walbaum) fry syndrome (RTFS) in England. Journal of Fish Disease 15: 203-6.

Aydin S, Ciltas A, Yetim H, Akyurt I. 2005. Clinical, pathological and haematological effects of Micrococcus luteus infections in rainbow trout (Oncorhynchus mykiss Walbaum). Journal of Animal and Veterinary Advances 4(2): 167-174.

Baird-Parker, A. C. (1965). The classification of Staphylococcus and Micrococcus from world-wide sources. Journal of General Microbiology 38:363-87.

Balashanmugam P and Kalaichelvan PT. 2015. Biosynthesis characterization of silver nanoparticles using Cassia roxburghii DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. International Journal of Nanomedicine 10(Suppl 1):87-97.

Baldellon C and Megraud F. 1985. Characterization of Micrococcaceae strains isolated from the human urogenital tract by the conventional scheme and a micromethod. Journal of Clinical Microbiology 21(3):474-

Bannerman TL, and Peacock S J. 2007. Staphylococcus, Micrococcus, and other catalase- positive cocci. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology (9th ed., pp. 390-404). Washington, USA: ASM Press.

Bari Q and Bhardwaj N. 2014. Degradation potential of Micrococcus luteus, Staphylococcus aureus and Serratia marcescens against some commonly used dyes. International Journal of Environmental Biology 42(2):5-145.

Beller HR, Goh EB, Keasling JD. 2010. Genes involved in long-chain alkene biosynthesis in Micrococcus luteus. Applied and Environmental Microbiology 76(4):1212-23.

Bevinakatti BG and Ninnekar HZ. 1993. Biodegradation of 4-chlorobiphenyl by Micrococcus species. World Journal of Microbiology and Biotechnology 9(5):607-8.

Bibel DJ and Smiljanic HJ. 1979. Interactions of Trichophyton mentagrophytes and Micrococcus on skin culture. Journal of Investigative Dermatology 72(3):133-7.

Biskupiak JE, Meyers E, Gillum AM, Dean,L, Trejo WH, Kiersch D R. 1988. Neoberninamycin, a new antibiotic produced by Micrococcus luteus. The Journal of Antibiotics, 41: 684-697

Blanc L, Castanier R, Mishra AK, Ray A, Besra GS, Sutcliffe I, Vercellone A, Nigou J. 2013. Gram-positive bacterial lipoglycans based on a glycosylated diacylglycerol lipid anchor are microbe-associated molecular patterns recognized by TLR2. Plos One 8(11).

Bultel-Poncé V, Debitus C, Berge J-, Cerceau C, Guyot M. 1998. Metabolites from the sponge-associated bacterium Micrococcus luteus. Journal of Marine Biotechnol 6(4):233-6.

Casida LE. 1980a. Death of Micrococcus luteus in soil. Applied and Environmental Microbiology 39(5):1031-4.

Casida LE. 1980b. Bacterial predators of Micrococcus luteus in soil. Applied and Environmental Microbiology 39(5):1035-41.

Ceotto H, Brede D, Salehian Z, Nascimento J, Fagundes PC, Nes IF, Bastos M. 2010. Aureocins 4185, bacteriocins produced by Staphylococcus aureus 4185: Potential application in food preservation. Foodborne Pathogens and Disease 7(10):1255-62.

CEPA. 1999. Prioritization of micro-organisms on the domestic substances list prior to the screening assessment under paragraph 74(b) of CEPA 1999. http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=19B91106-1 or http://www.ec.gc.ca/subsnouvelles-newsubs/19B91106-4767-4907-BA60-8FF16398251F/Prioritization%20Guidelines_EN.pdf.

CFIA. 2011. Organisms that do not require a plant protection permit to import. http://www.inspection.gc.ca

Chantawannakul P, Yoshimune K, Shirakihara Y, Shiratori A, Wakayama M, Moriguchi M. 2003. Crystallization and preliminary X-ray crystallographic studies of salt-tolerant glutaminase from Micrococcus luteus K-3. Acta Crystallographica Section D 59(3):566-8.

Chen H, Zhao G, Park D, Zhang Y, Xu L, Lee J, Kim C, Li W. 2009. Micrococcus endophyticus sp. nov., isolated from surface-sterilized Aquilaria sinensis roots. International Journal of Systematic and Evolutionary Microbiology 59(5):1070-5.

Chittpurna, Singh PK, Verma D, Pinnaka AK, Mayilraj S, Korpole S. 2011. Micrococcus lactis sp. nov., isolated from dairy industry waste. International Journal of Systematic and Evolutionary Microbiology 61(12):2832-6.

Cinquemani C, Boyle C, Bach E, Schollmeyer E. 2007. Inactivation of microbes using compressed carbon dioxide-an environmentally sound disinfection process for medical fabrics. The Journal of Supercritical Fluids 42(3):392-7.

Clark DJ, Hawrylik SJ, Kavanagh E, Opheim DJ. 2000. Purification and characterization of a unique alkaline elastase from Micrococcus luteus. Protein Expression and Purification 18(1):46-55.

Cohn, F. 1872. Untersuchungen über Bakterien. Beitr Biol Pflanz 1:127–244

de Lillo A, Quiros LM, Fierro JF. 1997. Relationship between antibacterial activity and cell surface binding of lactoferrin in species of genus Micrococcus. FEMS Microbiology Letters 150(1):89-94.

Dib J, Motok J, Zenoff VF, Ordonez O, Farias ME. 2008. Occurrence of resistance to antibiotics, UV-B, and arsenic in bacteria isolated from extreme environments in high-altitude (above 4400 m) Andean wetlands. Current Microbiolog 56:510–7

Dib JR, Liebl W, Wagenknecht M, Farías ME, Meinhardt F. 2013. Extrachromosomal genetic elements in Micrococcus. Applied Microbiology and Biotechnology 97(1):63-75.

Dimitrijević R, Stojanović M, Živković I, Petersen Ac, Jankov RM, Dimitrijević L, Gavrović-Jankulović M. 2009. The identification of a low molecular mass bacteriocin, rhamnosin A, produced by Lactobacillus rhamnosus strain 68. Journal of Applied Microbiology 107(6):2108-15.

DSMZ. 2015. BacDive: Micrococcus luteus (Schroeter 1872) Cohn 1872 emend. Wieser et al. DSM 20030. Leibniz Institute DSMZ - German collection of microorganisms and cell cultures BacDive - https://www.dsmz.de/catalogues/details/culture/dsm-20030.html

Eaton RW and Ribbons DW. 1982. Utilization of phthalate esters by Micrococcus. Archives of Microbiology 132(2):185-8.

Environment Canada. 2004. Guidance document for pathogenicity and toxicity of new microbial substances to aquatic and terrestrial organisms. Environmental Protection Service, Ottawa, ON, Report EPS 1/RM/44, 171 p. March 2004. http://publications.gc.ca/collections/Collection/En49-7-1-44E.pdf (Last viewed on August 12, 2015)

Environment Canada and Health Canada. 2011. Framework on the science-based risk assessment of micro-organisms under the Canadian Environmental Protection Act, 1999. http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=120842D5-1

Eucast. 2015 European committee on antimicrobial susceptibility testing breakpoint tables for interpretation of MICs and zone diameters Version 5.0, valid from 2015-01-01. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf

Euzeby J P. 1997. "List of bacterial names with standing in nomenclature: a folder available on the Internet". International Journal of Systematic Bacteriology 47 (2): 590–2. http://www.bacterio.net

Faison BD, Cancel CA, Lewis SN, Adler HI. 1990. Binding of dissolved strontium by Micrococcus luteus. Applied and Environmental Microbiology 56(12):3649-56.

Fazlani S, Abubakar M, Shah S, ul Hassan M, Arshed M. 2008. Bio-morphological characteristics of bacterial species identified from mastitic milk samples of camel. The Internet Journal of Veterinary Medicine 6(1).

Fischer-Colbrie G, Matama T, Heumann S, Martinkova L, Cavaco Paulo A, Guebitz G. 2007. Surface hydrolysis of polyacrylonitrile with nitrile hydrolysing enzymes from Micrococcus luteus BST20. Journal of Biotechnology 129(1):62-8.

Fleming A. 1922. On a remarkable bacteriologic element found in tissues and secretions. Proceedings of Royal Society. London. Series B, Biological Sciences 93:12-306.

Fujishima T and YamamotoY. 1986. Process for producing ribavirin. US Patent No: 4,614,719. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=4,614,719.PN.&OS=PN/4,614,719&RS=PN/4,614,719

Ganz T, Gabayan V, Liao HI, Liu L, Oren A, Graf T, Cole AM. 2003. Increased inflammation in lysozyme M-deficient mice in response to Micrococcus luteus and its peptidoglycan. Blood 101(6):2388-92.

García Fontán MC, Lorenzo JM, Parada A, Franco I, Carballo J. 2007. Microbiological characteristics of “androlla”, a spanish traditional pork sausage. Food Microbiology 24(1):52-8.

Gorsuch EL, Grinshpun SA, Willeke K, Reponen T, Moss CE, Jensen PA. 1998. Method for evaluating germicidal ultraviolet inactivation of biocontaminated surfaces. International Journal of Occupational Safety and Ergonomics 4:287-98.

Greenblatt CL, Baum J, Klein BY, Nachshon S, Koltunov V, Cano RJ. 2004. Micrococcus luteus-survival in amber. Microbial Ecology 48(1):120-7.

Gu J. 2007. Microbial colonization of polymeric materials for space applications and mechanisms of biodeterioration: A review. International Biodeterioration & Biodegradation 59(3):170-9.

Gürel HG, Basciftci FA, Arslan U. 2009. Transient bacteremia after removal of a bonded maxillary expansion appliance. American Journal of Orthodontics and Dentofacial Orthopedics 135(2):190-3.

Herrmann P and Klein P. 1999. Method of preparing lysozyme dimers. US Patent No: 5,989,880. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5,989,880.PN.&OS=PN/5,989,880&RS=PN/5,989,880

Hillesland KL, Lenski RE, Velicer GJ. 2007. Ecological variables affecting predatory success in Myxococcus xanthus. Microbial Ecology 53(4):571-8.

Hirai Y. 1991. Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. The Journal of Hospital Infection 19(3):191-200.

Hirata Y, Sata M, Makiuchi Y, Morikane K, Wada A, Okabe N, Tomoike H. 2009. Comparative analysis of Micrococcus luteus isolates from blood cultures of patients with pulmonary hypertension receiving epoprostenol continuous infusion. Journal of Infection and Chemotherapy : Official Journal of the Japan Society of Chemotherapy 15(6):424-5.

HSE-UK, 2015. Health and Safety Executive of United Kingdom. http://www.hse.gov.uk/pubns/misc208.pdf

Iketani M, Nishimura H, Akayama K, Yamano Y, Morishima I. 1999. Minimum structure of peptidoglycan required for induction of antibacterial protein synthesis in the silkworm, Bombyx mori. Insect Biochemistry and Molecular Biology 29(1):19-24.

Kanost MR, Dai W, Dunn PE. 1988. Peptidoglycan fragments elicit antibacterial protein synthesis in larvae of Manduca sexta. Archives of Insect Biochemistry and Physiology 8(3):147-64.

Kao C, Chiang C, Huang J. 2014. Micrococcus species-related peritonitis in patients receiving peritoneal dialysis. International Urology and Nephrology 46(1):261-4.

Kaprelyants AS and Kell DB. 1993. Dormancy in stationary-phase cultures of Micrococcus luteus: Flow cytometric analysis of starvation and resuscitation. Applied and Environmental Microbiology 59(10):10-3187.

Kaprelyants AS, Gottschal JC, Kell DB. 1993. Dormancy in non-sporulating bacteria. FEMS Microbiology Letters 104(3-4):271-86.

Khan TS and Rind R. 2001. Isolation and characterization of bacterial species from surgical and non-surgical wounds located on body surface of buffaloes, cattles, sheep and goats. Pakistan Journal of Biological Sciences 4(6):696-702.

Kloos WE, Tornabene TG, Schleifer KH. 1974. Isolation and characterization of Micrococcus from human skin, including two new species: Micrococcus lylae and Micrococcus kristinae. International Journal of Systematic Bacteriology 24(1):79-101.

Kloos WE and Musselwhite MS. 1975. Distribution and persistence of Staphylococcus and Micrococcus species and other aerobic bacteria on human skin. Applied Microbiology 30(3):381-5.

Kloos W E, Zimmerman R J, Smith R F. 1976. Preliminary studies on the characterization and distribution of Staphylococcus and Micrococcus species on animal skin. Applied and Environmental Microbiology 31:53–9.

Kocur M, Kloos W, Schleifer K. 2006. The genus Micrococcus. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt (Eds.), The Prokaryotes (pp. 961-71): Springer New York.

Kooken JM, Fox KF, Fox A. 2012. Characterization of Micrococcus strains isolated from indoor air. Molecular and Cellular Probes 26(1):1-5.

Królasik J, Zakowska Z, Krępska M, Klimek L. 2010. Resistance of bacterial biofilms formed on stainless steel surface to disinfecting agent. Polish Journal of Microbiology 59, 281-7.

Lampert Y, Kelman D, Dubinsky Z, Nitzan Y, Hill RT. 2006. Diversity of culturable bacteria in the mucus of the red sea coral Fungia scutaria. FEMS Microbiology Ecology 58:10-99.

Leung, K., Trevors, J.T. and Lee, H. 1995. Survival of and lacZ expression in recombinant Pseudomonas strains introduced into river water microcosms. Can. Journal of Microbiology 41: 461-469.

Levchenko LA, Sadkov AP, Lariontseva NV, Koldasheva EM, Shilova AK, Shilov AE. 2002. Gold helps bacteria to oxidize methane. Journal of Inorganic Biochemistry 88(3–4):251-3.

Liebl W, Kloos WE, Ludwig W. 2002. Plasmid-borne macrolide resistance in Micrococcus luteus. Microbiology (Reading, England) 148(Pt 8):2479-87.

Liu H, Xu Y, Ma Y, Zhou P. 2000. Characterization of Micrococcus antarcticus sp. nov., a psychrophilic bacterium from Antarctica. International Journal of Systematic and Evolutionary Microbiology 50(2):715-9.

Liu X, Wang B, Jiang C, Liu S. 2007. Micrococcus flavus sp. nov., isolated from activated sludge in a bioreactor. International Journal of Systematic and Evolutionary Microbiology 57(1):66-9.

Lo Giudice A, Bruni V, Michaud L. 2007. Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. Journal of Basic Microbiology 47(6):496-505.

López L, Pozo C, Rodelas B, Calvo C, Juárez B, Martínez-Toledo MV, González-López J. 2005. Identification of bacteria isolated from an oligotrophic lake with pesticide removal capacities. Ecotoxicology 14:14-299.

LPSN. 2015. List of procaryotic names with standing in nomenclature. http://www.bacterio.net/micrococcus.html

Magee JT, Burnett IA, Hindmarch JM, Spencer RC. 1990. Micrococcus and Stomatococcus spp. from human infections. Journal of Hospital Infection 16(1):67-73.

Malic S, Hill KE, Playle R, Thomas DW, Williams DW. 2011. In vitro interaction of chronic wound bacteria in biofilms. Journal of Wound Care:20: 569-77.

Manikandan M, Kannan V, Pasic L. 2011. Extraction, purification and characterization of a protease from Micrococcus sp. VKMM 037. Environmental Technology 32:1487–95.

Marinella MA, Pierson C, Chenoweth C. 1997. The stethoscope: a potential source of nosocomial infection? Archives of Internal Medicine 157(7):786-90.

Martin DJH, Denyer SP, McDonnell G, Maillard JY. 2008. Resistance and cross-resistance to oxidising agents of bacterial isolates from endoscope washer disinfectors. Journal of Hospital Infection 69(4):377-83.

Miltiadous G and Elisaf M. 2011. Native valve endocarditis due to Micrococcus luteus: A case report and review of the literature. Journal of Medical Case Reports 29:251.

Monodane T, Kawabata Y, Yang S, Hase S, Takada H. 2001. Induction of necrosis factor-alpha and interleukin-6 in mice in vivo and in murine peritoneal macrophages and human whole blood cells in vitro by Micrococcus luteus teichuronic acids. Journal of Medical Microbiology 50(1):4-12.

Mortensen N and Kocur M. 1967. Correlation of DNA base composition and acid formation from glucose of Staphylococcus and Micrococcus. Acta Pathologica Microbiologica Scandinavica 69(3):445-57.

Mukamolova GV, Kaprelyants AS, Kell DB. 1995. Secretion of an antibacterial factor during resuscitation of dormant cells in Micrococcus luteus cultures held in an extended stationary phase. Antonie Van Leeuwenhoek 67:289-95

Mukamolova GV, Turapov OA, Kazarian K, Telkov M, Kaprelyants AS, Kell DB, Young M. 2002. The rpf gene of Micrococcus luteus encodes an essential secreted growth factor. Molecular Microbiology 46(3):611-21.

Mukamolova GV, Murzin AG, Salina EG, Demina GR, Kell DB, Kaprelyants AS, Young M. 2006. Muralytic activity of Micrococcus luteus rpf and its relationship to physiological activity in promoting bacterial growth and resuscitation. Molecular Microbiology 59(1):84-98.

NCBI. 2015. NCBI taxonomy. http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1270

Oka N, Hartel PG, Finlay-Moore O, Gagliardi J, Zuberer DA, Fuhrmann JJ, Angle JS, Skipper HD. 2000. Misidentification of soil bacteria by fatty acid methyl ester (FAME) and BIOLOG analyses. Biology and Fertiityl of Soils 32(3):256-8.

Ordonez OF, Flores MR, Dib JR, Paz A, Farias ME. 2009. Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. Microbial Ecology 58:461-73.

Panneerselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK. 2005. Synthesis of schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. European Journal of Medicinal Chemistry 40(2):225-9.

Panneerselvam P, Rather BA, Ravi Sankar Reddy D, Ramesh Kumar N. 2009. Synthesis and anti-microbial screening of some schiff bases of 3-amino-6, 8-dibromo-2-phenylquinazolin-4 (3 H)-ones. European Journal of Medicinal Chemistry 44(5):2328-33.

Payne JH, Welch JC, Vora AJ. 2003. Fatal pulmonary hemorrhage associated with micrococcal infection in two children with acute lymphoblastic leukemia. Journal of Pediatric Hematology/Oncology 25(12):969-74.

Peces R, Gago E, Tejada F, Laures AS, Alvarez-Grande J. 1997. Relapsing bacteraemia due to Micrococcus luteus in a haemodialysis patient with a perm-cath catheter. Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association 12(11):2428-9.

PHAC. 2011. Pathogen Safety Data Sheet - Infectious Substances, Micrococcus. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/micrococcus-eng.php

Piersen CE, Prince MA, Augustine ML, Dodson ML, Lloyd RS. 1995. Purification and cloning of Micrococcus luteus ultraviolet endonuclease, an N-glycosylase/abasic lyase that proceeds via an imino enzyme-DNA intermediate. The Journal of Biological Chemistry 270(40):23475-84.

Pittet V, Haakensen M, Ziola B. 2010. Rapid screening for gram-negative and gram-positive beer-spoilage firmicutes using a real-time multiplex PCR. Journal of the American Society of Brewing Chemists 68(2):89.

Powell S, Perry J, Meikle D. 2003. Microbial contamination of non-disposable instruments in otolaryngology out-patients. The Journal of Laryngology & Otology 117(02):122-5.

Prado B, Jara A, del Moral A, Sánchez E. 2001. Numerical taxonomy of microorganisms isolated from goat cheese made in Chile. Current Microbiology 43(6):396-9.

Prakash O, Nimonkar Y, Munot H, Sharma A, Vemuluri VR, Chavadar MS, Shouche YS. 2014. Description of Micrococcus aloeverae sp. nov., an endophytic actinobacterium isolated from Aloe vera. International Journal of Systematic and Evolutionary Microbiology 64(Pt 10):3427-33.

Purmal K, Chin S, Pinto J, Yin W, Chan K. 2010. Microbial contamination of orthodontic buccal tubes from manufacturers. International Journal of Molecular Sciences 11(9):3349-56.

Puyen ZM, Villagrasa E, Maldonado J, Diestra E, Esteve I, Solé A. 2012. Biosorption of lead and copper by heavy-metal tolerant Micrococcus luteus DE2008. Bioresource Technology 126:233-7.

Rakhashiya PM, Patel PP, Thaker VS. 2016. Whole genome sequences and annotation of Micrococcus luteus SUBG006, a novel phytopathogen of mango. Genomics Data 6:10-1.

Ramos M, Hachem R, Youssef S, Fang X, Jiang Y, Raad I. 2009. The crucial role of catheters in micrococcal bloodstream infections in cancer patients. Infection Control and Hospital Epidemiology 30(1):83-5.

Raza F and Faisal M. 2013. Growth promotion of maize by desiccation tolerant Microccocus luteus-chp37 isolated from Cholistan desert, Pakistan. Australian Journal of Crop Science 7(11):1693-8.

Rickard AH, McBain AJ, Ledder RG, Handley PS, Gilbert P. 2003. Coaggregation between freshwater bacteria within biofilm and planktonic communities. FEMS Microbiology Letters 220(1):133-40.

Rieser G, Scherer S, Wenning M. 2013. Micrococcus cohnii sp. nov., isolated from the air in a medical practice. International Journal of Systematic and Evolutionary Microbiology 63(Pt 1):80-5.

Rosypal S, Rosypalová A, Horejs J. 1966. The classification of Micrococcus and Staphylococcus based on their DNA base composition and adansonian analysis. Journal of General Microbioogy. 44:281-92.

Rusin PA, Rose JB, Gerba CP. 1997. Health significance of pigmented bacteria in drinking water. Water Science and Technology 35(11):21-7.

Seifert H, Kaltheuner M, Perdreau-Remington F. 1995. Micrococcus luteus endocarditis: Case report and review of the literature. Zentralblatt Für Bakteriologie 282(4):431-5.

Sezen K, Demir I, Kati H, Demirbag Z. 2005. Investigations on bacteria as a potential biological control agent of summer chafer, Amphimallon solstitiale L. (coleoptera: Scarabaeidae). Journal of Microbiology (Seoul, Korea) 43(5):463-8.

Shanks D, Goldwater P, Pena A, Saxon B. 2001. Fatal Micrococcus sp. infection in a child with leukaemia- a cautionary case. Medical and Pediatric Oncology 37(6):553-4.

Shapo JL, Moeller PD, Galloway SB. 2007. Antimicrobial activity in the common seawhip, Leptogorgia virgulata (cnidaria: Gorgonaceae). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 148(1):65-73.

Shi Y and Zhou C. 2011. Synthesis and evaluation of a class of new coumarin triazole derivatives as potential antimicrobial agents. Bioorganic & Medicinal Chemistry Letters 21(3):956-60.

Shingade SG and Bari SB. 2013. Synthesis and antimicrobial screening of 4-thiazolidinone and 2-azetidinone derivatives of piperazine. Medicinal Chemistry Research 22(2):699-706.

Sims GK, Sommers LE, Konopka A. 1986. Degradation of pyridine by Micrococcus luteus isolated from soil. Applied and Environmental Microbiology 51(5):963-8.

Sims GK and O'Loughlin EJ. 1992. Riboflavin production during growth of Micrococcus luteus on pyridine. Applied and Environmental Microbiology 58(10):3423-5.

Smith KJ, Neafie R, Yeager J, Skelton HG. 1999. Micrococcus folliculitis in HIV-1 disease. British Journal of Dermatology 141:558-61.

Stackebrandt E, Koch C, Gvozdiak O, Schumann P. 1995. Taxonomic dissection of the genus Micrococcus: Kocuria gen. nov., Nesterenkonia gen. nov., Kytococcus gen. nov., Dermacoccus gen. nov., and Micrococcus Cohn 1872 gen. emend. International Journal of Systematic Bacteriology 45(4):682-92.

StrainInfo. 2014. Strain Passport ATCC 4698 Micrococcus luteus. www.straininfo.net

Sugita H, Shibuya K, Shimooka H, Deguchi Y. 1996. Antibacterial abilities of intestinal bacteria in freshwater cultured fish. Aquaculture 145(1):195-203.

SINTEF. 2013. Super sunscreen from fjord bacteria. ScienceDaily. ScienceDaily, 6 August 2013. http://www.sciencedaily.com/releases/2013/08/130806091556.htm

Tagg JR, Chilcott CN, Alqumber MAA. 2006a. Skin treatment compositions. Canadian Patent No: 2602108. http://brevets-patents.ic.gc.ca/opic-cipo/cpd/eng/patent/2602108/summary.html?type=number_search&tabs1Index=tabs1_1

Tagg JR, Chilcott CN, Alqumber MAA. 2006b. Skin treatment compositions.WIPO International Publication No. WO2006104403 A1. Skin treatment compositions. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2006104403

Tagg JR, Chilcott CN, Alqumber MAA. 2012. Skin treatment compositions. US Patent No: 8,283,136. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8,283,136.PN.&OS=PN/8,283,136&RS=PN/8,283,136

Tambekar DH, Gulhane PB, Dahikar SG, Dudhane MN. 2008. Nosocomial hazards of doctor's mobile phones in hospitals. Journal of Medical Science, 8(1):73-6.

Tang JS and Gillevet PM. 2003. Reclassification of ATCC 9341 from Micrococcus luteus to Kocuria rhizophila. International Journal of Systematic and Evolutionary Microbiology 53(Pt 4):995-7.

Tiago I, Chung AP, Veríssimo A. 2004. Bacterial diversity in a nonsaline alkaline environment: Heterotrophic aerobic populations. Applied and Environmental Microbiology 70(12):7378-87.

US-FDA. 2006. Hanford pharmaceuticals issues a nationwide recall of cefazolin for injection because of possible health risk. http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2006/ucm111963.htm

Vandecandelaere I, Matthijs N, Nelis HJ, Depuydt P, Coenye T. 2013. The presence of antibiotic-resistant nosocomial pathogens in endotracheal tube biofilms and corresponding surveillance cultures. Pathogens and Disease 69(2):142-8.

Van Hoey-De-Boer KlA and Hageman RJJ. 1999. Probiotic nutritional preparation. European Patent No: EP 0 904 784 A1. https://data.epo.org/publication-server/pdf-document?cc=EP&pn=0904784&ki=A1

Van Trappen S, Mergaert J, Van Eygen S, Dawyndt P, Cnockaert MC, Swings J. 2002. Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. Systematic and Applied Microbiology 25(4):603-10.

van Veen, J.A., van Overbeek, L.S. and van Elsas, J.D. 1997. Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews 61: 121-135.

Vermin RJC and Spinnler HE. 2004. Dairy product and method for preparing same. US Patent No: 6,753,022. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=6,753,022.PN.&OS=PN/6,753,022&RS=PN/6,753,022

Verslyppe B, De Smet W, De Baets B, De Vos P, Dawyndt P. 2014. StrainInfo introduces electronic passports for microorganisms. Systematic and Applied Microbiology 37(1):42-50.

Votyakova TV, Kaprelyants AS, Kell DB. 1994. Influence of viable cells on the resuscitation of dormant cells in Micrococcus luteus cultures held in an extended stationary phase: The population effect. Applied and Environmental Microbiology 60(9):3284-91.

Wagenknecht M, Dib JR, Thurmer A, Daniel R, Farias ME, Meinhardt F. 2010. Structural peculiarities of linear megaplasmid, pLMA1, from Micrococcus luteus interfere with pyrosequencing reads assembly. Biotechnology Letters 32(12):1853-62.

Wieser M, Denner EB, Kampfer P, Schumann P, Tindall B, Steiner U, Vybiral D, Lubitz W, Maszenan AM, Patel BK, et al. 2002. Emended descriptions of the genus Micrococcus, Micrococcus luteus (Cohn 1872) and Micrococcus lylae (Kloos et al. 1974). International Journal of Systematic and Evolutionary Microbiology 52(Pt 2):629-37.

Xylander WER and Nevermann L. 1990. Antibacterial activity in the hemolymph of myriapods (arthropoda). Journal of Invertebrate Pathology 56(2):206-14.

Yokozeki K, Majima E, Hirose Y, Sato T, Yukawa T. 1987. Process for the production of L-aspartyl-L-phenylalanine esters. US Patent No: 4,666,838. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=4,666,838.PN.&OS=PN/4,666,838&RS=PN/4,666,838

Yokozeki K, Tsuji T, Izawa K. 2001. Process for preparing 2,6-diaminopurine-2'-deoxyriboside and 2'-deoxyguanosine. US Patent No: 6,197,552. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=6,197,552.PN.&OS=PN/6,197,552&RS=PN/6,197,552

Young M, Artsatbanov V, Beller HR, Chandra G, Chater KF, Dover LG, Goh E, Kahan T, Kaprelyants AS, Kyrpides N, et al. 2010. Genome sequence of the

Fleming strain of Micrococcus luteus, a simple free-living Actinobacterium. Journal of Bacteriology 192(3):841-60.

Zhang J, Liu X, Liu S. 2010. Agrococcus terreus sp. nov. and Micrococcus terreus sp. nov., isolated from forest soil. International Journal of Systematic and Evolutionary Microbiology 60(8):1897-903.

Zhao G, Li J, Qin S, Zhang Y, Zhu W, Jiang C, Xu L, Li W. 2009. Micrococcus yunnanensis sp. nov., a novel Actinobacterium isolated from surface-sterilized Polyspora axillaris roots. International Journal of Systematic and Evolutionary Microbiology 59(10):2383-7.

Zheng C, Qu B, Wang J, Zhou J, Wang J, Lu H. 2009. Isolation and characterization of a novel nitrobenzene-degrading bacterium with high salinity tolerance: Micrococcus luteus. Journal of Hazardous Materials 165(1–3):1152-8.

Zherebtsov SV and Tomilin NV. 1975. Postreplication DNA repair in ultraviolet-irradiated Micrococcus luteus. Biochimica et Biophysica Acta 383(1):16-22.

Zhuang WQ, Tay JH, Maszenan AM, Tay ST. 2003. Isolation of naphthalene-degrading bacteria from tropical marine sediments. Water Science Technology 47:303-8.

Appendices

Appendix A: Phenotypic characteristics of M. luteus strain ATCC 4698

Table A-1: Phenotypic characteristics of M. luteus strain ATCC 4698 and K. rhizophila ATCC 9341[a]

Characteristic	M. luteus strain ATCC 4698	K. rhizophila strain ATCC 9341
Oxidase test	+	-
Simmons' citrate test	-	+
Acid production from glucose	-	+
Acid production from fructose	-	+
Major fatty acids	ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{15:0}$

[a] Data compiled from Tang and Gillevet, 2003
+ indicates a positive reaction
- indicates a negative reaction

Appendix B: Genotypic characteristics of M. luteus strain ATCC 4698

Table B-1: Plasmid-associated phenotypes in Micrococcus species[a]

Plasmid name and size (kb)	Source of strain(s)	Associated phenotype
Unnamed (9 plasmids, 1 – 30.2)	Not described (multiple strains)	Cryptic
Unnamed	Not described	Multiple antibiotic resistance and heavy metals resistance
pMQV10, 10	Not described	Streptomycin resistance, cholesterol degradation
Unnamed	Soil	Nalidixic acid resistance, degradation of malathion and chlorpyrifos
Unnamed	Soil	Degradation of malathion and chlorpyrifos
pSD10, 50.7	Coastal marine sediments	Transposases, replication genes
pMEC2, 4.2	Human skin	Macrolide and lincosamide resistance
pMLU1, 2.3	Soil	Cryptic
Unnamed	Lake water	Osmotolerance
Unnamed, ca. 2.8	Estuary water and sediments	Resistance to kanamycin, tetracycline, erythromycin, ampicillin, tobramycin, streptomycin, rifampicin, and chloramphenicol
Unnamed	Poultry litter	resistance to penicillin, ampicillin, tetracycline, amoxicillin, kanamycin and chloramphenicol
Unnamed	Grape stem and leaves	Not described
Unnamed	Petroleum-contaminated soil	Hydrocarbon biodegradation
pLMA1, 110	Lake water	Transposases (Wagenknecht et al. 2010, Erythromycin resistance
pLMH5, 110	Lake water	Not determined
pLMV7, 90	Lake water	Not determined
pLMA7, 80	Lake water	Not determined

[a] Data compiled from Dib et al. 2013 unless referenced otherwise.

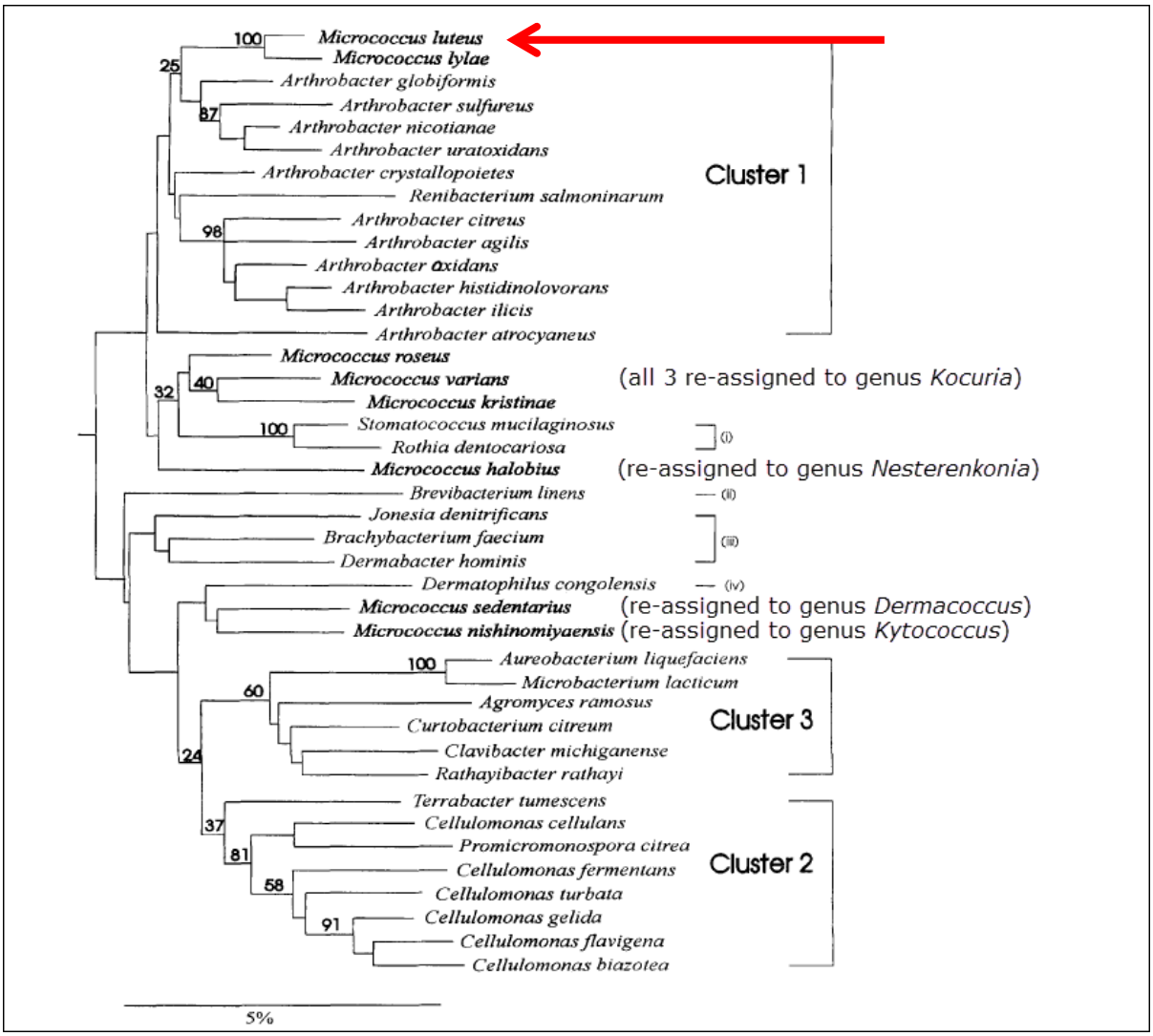

Figure B-1: Phylogenetic tree of Micrococcus species within the Arthrobacter lineage of the subphylum of Actinomycetales, based on 16S rRNA gene sequence analysis (Stackebrandt et al. 1995). The arrow indicates the DSL strain.