SVU- International Journal of Veterinary Sciences, 5(2): 77-90, 2022
Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

Incidence of *Chryseobacterium spp.* in raw milk and some dairy products

Amal S. Mohamed[1*], Laila M. El-Malt[2], Karima G. Abdel Hameed[2], and Mona A. El-Zamkan[2]

[1]A post-graduate researcher, Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt, [2] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt.

Abstract

A total of 225 random samples of raw cow milk (150), Kareish cheese, Domiati cheese and butter (25 samples each), were collected from various farmers' houses, dairy shops, street vendors and supermarkets located at Aswan city, Egypt, to be examined by culture and molecular methods in which 16S rRNA gene was used; the obtained PCR product was then purified and sequenced to detect the existence of *Chryseobacterium* spp. All cheese and butter samples didn't show the characteristic colonies of *Chryseobacterium* spp., while two raw cow milk samples exhibited the characteristic *Chryseobacterium* colonies. The suspected *Chryseobacterium* spp. were confirmed by PCR and DNA sequencing and comparing the sequenced DNA with those on GenBank using the BLAST tool on GenBank, underlining its occurrence in raw cow milk samples with an incidence of 1.3%. The isolated strains showed the highest similarity with *Chryseobacterium jip* 108/83 (100%). According to obtained data and our knowledge, it is the first time to isolate *Chryseobacterium* spp. from raw milk in Egypt. The isolated strains were registered on the GenBank with the proposed names *Chryseobacterium* Egyam strain AM1 (Accession No. MK059947) and *Chryseobacterium* Egyam strain AM2 (Accession No. MK059948). A constructed phylogenetic tree by using the neighbour-joining method was applied to detect the exact taxonomic position of the obtained strains.

Keywords:
Chryseobacterium spp., Milk, 16S rRNA

DOI: 10.21608/svu.2022.114720.1170 **Received:** January 12, 2022 **Accepted**: May 27, 2022 **Published**: June 30, 2022 ***Corresponding author:** Amal S. Mohamed **E-mail:** amhnl2017@gmail.com

Citation: Mohamed et al., Incidence of *Chryseobacterium spp.* in raw milk and some dairy products. SVU-IJVS 2022, 5(2): 77-90.

Competing interest: The authors have declared that no competing interest exists.

Introduction

Raw milk is an example of an environment that contains a diverse and complex microbial population (Quigley et al., 2011 and Vacheyrou et al., 2011). The extended duration of storage of raw milk at low temperatures creates selective conditions that promote the growth of psychrotrophic bacteria, altering the microbial composition of the milk (Xin et al., 2017). Amongst psychrotrophic bacteria that are frequently isolated from raw milk is *Flavobacterium* (Vithanage et al., 2016 and Yuan et al., 2017). During the reclassification of the members of this genus based on rRNA cistron similarity studies, the genus *Chryseobacterium* was introduced and described for first time by Vandamme et al. (1994). Higher resolutions achieved by 16S rRNA gene sequencing than old methods such as DNA-rRNA hybridization technique that was extensively used for phylogenetic investigations of the genus *Flavobacterium* and related genera during the 1980s and 1990s (Bauwens and De Ley, 1981; Segers et al., 1993; Mudarris et al., 1994; Vandamme et al., 1994; Vancanneyt et al., 1996).

Many *Chryseobacterium* strains occur in soil, fresh water and marine environments, while others are found in dairy products. Several *Chryseobacterium spp*. are associated with the spoilage of dairy products during cold storage like *C. balustinum*, *C. gleum* and *C. joostei* (Bernardet et al., 2002). *Chryseobacterium* spp. also produce off- odors in pasteurized milk and cream (García-López et al., 1999), surface taint and apple odor in butter, thinning in creamed rice and bitterness (Jooste and Hugo, 1999 and Bernardet et al., 2002). Spoilage caused by this bacterium is mainly contributed to the production of heat stable lipases and proteases (Bernardet et al., 2002 and Yuan et al., 2018). *Chryseobacterium* as psychrotrophic bacteria present more serious challenges to the dairy industry. From the public health point of view, *Chryseobacterium* are opportunistic pathogens of humans and animals and have been described as an etiological agent of meningitis, bacteremia, pneumonia,endocarditis, tracheobronchitis, infections of skin and soft tissue, ocular infections, urinary tract infection, pyonephrosis, dental plaque (Bloch et al., 1997; Booth, 2014; Lo and Chang, 2014; Omar et al., 2014; Virok et al., 2014; Brkic et al., 2015; Garg et al., 2015 and Abdalhamid et al., 2016). Most common strains isolated from clinical cases were *C. meningiosepticum*, *C. Indologenes* and *C. gleum* (Imataki and Uemura 2016; Rajendran et al., 2016; Saleem et al., 2016; González-Castro et al., 2017; Jain et al., 2017 and Rawat et al., 2017; Lin et al., 2018 and Bhalla et al., 2018).

This study aimed to the detection and phenotypic description of *Chryseobacterium* spp. in raw milk and some milk products collected from Aswan city, Egypt, identification of the isolated *Chryseobacterium* spp. by *16S rRNA* gene sequencing using Universal Method, phylogenetic tree analysis of the sequenced isolates.

Materials and methods

From various dairy places at Aswan city, Egypt, a total of 150 random samples of raw cow's milk and additionally, 25 locally manufactured milk product samples were collected from each of the following categories Domiati, Kareish cheese and butter. Collected samples were transferred in an ice box directly to the laboratory with a minimum of delay to be immediately examined for the presence of *Chryseobacterium* spp.

Isolation and identification of *Chryseobacterium* spp.

Preparation of samples

Raw milk samples were examined for heat treatment according to Lampert

(1975), cheese samples prepared as described by ISO 8261 (2001). Briefly, 10 gm of each sample were transferred to sterile containers and homogenized with 90 ml of Maximum Recovery Diluent (Oxoid, CM0733) solution preheated to 45 °C and thoroughly mixed until the cheese is thoroughly dispersed. butter samples prepared by melting of 10 gm in 45 °C water bath, centrifuged until the serum is separated.

Cultural condition:

One tenth ml of raw cow's milk, prepared cheese and butter serum solutions were carefully transferred into each of the appropriately marked plates of Standard Plate Count agar (SPC) (Oxoid, CM0463) as described by Hantsis-Zacharov and Helpern (2007) with slight change where plates were incubated 2 days at 25 °C followed by 2 other days at 7 °C. Creamy, yellow, and orange colonies picked up and enriched in nutrient broth for 2 days at 7 ºC then sub-cultured on SPC agar plates for further identification. Suspected colonies were identified phenotypically by Gram stain, oxidase (Oxoid MB0266), catalase (Land et al., 1991), API 20 NE (Biomerieux) including conventional and assimilation testes, and flexirubin pigment production by using of KOH (20%) according to method described by Fautz and Reachard (1980).

Molecular confirmation of presumptive *Chryseobacterium* isolates

DNA extraction

DNA extraction was done using QIAamp DNA mini kit (Qiagen, Germany) according to the manufacture's recommendations.

Amplification and sequencing 16S rRNA

Amplification of 16S rRNA gene was done using the universal primers F27(5'-AGAGTTTGATCMTGGCTCAG-3') and R1492(5'-TACGGYTACCTTGTTACGACTT-3') which targeted to universally conserved regions and permit the amplification to 1485 bp fragments (Lagacé et al., 2004). PCR amplification was carried out in T3 thermal cycler (Biometra). Primers were utilized in 25 µl reaction including,12.5 µL of Emerald Amp GT PCR master mix (2x premix) (Takara). 1 µL of each primer, 4.5 µL of water, and 6 µL of DNA template. Initial DNA denaturation and enzyme activation steps were proceeded at 94 ºC for 15 min., followed by 35 cycles of denaturation at 94 ºC for 30 s., annealing at 56 ºC for 1 min. and extension at 72 ºC for 1 min., and a final extension at 72ºC for 10 min. The PCR products (16S rRNA gene) were separated by electrophoresis on 1% agarose gel (Applichem GmbH, Darmstadt, Germany) in 1× TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 20 µL of the products was loaded in each gel slot to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra, Göttingen, Germany) and the data was analyzed through computer software. PCR products were purified using QIAquick PCR product extraction kit (Qiagen Inc. Valencia CA).

For sequence reaction, Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) used, using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Thermo Fisher Scientific, USA), with (Cat. No. 4336817). And then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer, USA. The obtained 16S rRNA gene sequences were submitted to the genebank (https://blast.ncbi.nlm.nih.gov/Blast.cgi.)

The sequence alignment was performed using BIOEDIT (Version 7.0.9.0, Hall, 1999) software. The Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) was used to complete

sequencing alignment between the sequences of the isolates and those deposited in the GenBank DNA database.

Phylogenetic analysis

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software Pairwise, which was designed by Thompson et al., (1994). Phylogenetic comparisons of the aligned sequences for the gene were also performed with the MegAlign module of Lasergene DNA Star software to determine nucleotide and amino acid sequence similarities and relationships.

Results and Discussion

According to Hantsis-Zacharov and Helpern (2007); Hantsis-Zacharov et al., (2008a, 2008b); and Bekker (2011), the focus in this study was on Gram negative yellow and/or orange colonies with circular edges, after aerobic plating on SPC agar and incubated at 25 ºC for 4 days and, oxidase and catalase positive colonies. Results presented in Table 1 and Fig. 1 illustrated the incidence of the examined samples that potentially contaminated with *Chryseobacterium* spp. It revealed that 10 out of 225 (20%) raw milk and milk products samples were contaminated with yellow, gram negative, oxidase and catalase positive colonies which suspected to be *Chryseobacterium* spp. The targeted colonies existed in 7% of the investigated raw cow milk samples, while the rest of the examined samples did not show the targeted colonies (Kareish and Domiati Cheese and butter), so the total percent of positive suspected colonies is 4.4%. The yellow colonies (Fig .2A) are shown in Fig.1.

Flexirubins are the unique type of bacterial pigments that produced by *Chryseobacterium* spp., *Cytophaga* spp. and *Flexibacter* spp. (Krebs et al., 2012 & 2013; Venil et al., 2014 & 2015). The presence of flexirubin pigments was investigated by flooding the plates with 20% (w/v) potassium hydroxide (Fautz and Reichenbach, 1980) and color change from gold to red-brown indicates the presence of flexirubin as shown in Fig 2B.

In the current study, flexirubin pigment found to be produced by 2 (20%) out of 10 isolates (Table 1 and Fig. 1). Similar finding was obtained by Kim et al., (2005), Hantsis -Zacharov et al., (2008 b), Chaudhari et al., (2009), Bekker (2011) and Ahmad et al., (2012) where the flexirubin-type pigment was detected in different *Chryseobacterium* spp. according to this method. As it is difficult to isolate and confirm *Chryseobacterium* spp, using the conventional culture and PCR methods, so we recommend the use of flexirubin pigment as a vital step to select the isolates to detect the differential phylogenetic characteristic of *Chryseobacterium* spp. as this pigment works as a biomarker for the bacterial group that produces it in which *Chryseobacterium* spp. falls and donating it a specific characteristic.

Chryseobacterium spp. is nonfermenting Gram-negative bacilli, has clinical importance in nosocomial infections (Hantsis-Zacharov et al., 2008a and de Carvalho Filho et al., 2017), and its identification is a challenge to microbiology laboratories. As a result of the variation and diversity between conventional tests (phenotypic, biochemical and API test) of the potentially *Chryseobacterium* spp. strains obtained in this study, the two isolates were subjected for further identification by16S rRNA gene sequencing for greater reliability of identification. Identification based on the 16S rRNA, is considered a valid molecular chronometer which is essential for a precise assessment of phylogenetic relatedness of organisms because 16S rRNA, is an ~1500

base pair gene coding for a catalytic RNA that is part of the 30S ribosomal subunit exists universally among prokaryotic cells (bacteria) and conserved and variable sequences regions evolving at very different rates, critical for the concurrent universal amplification and measurement of both close and distant phylogenetic relationships. These characteristics allow the use of 16S rRNA in the assignment of close relationships at the genus (Clarridge, 2004) and in many cases at the species level (Conlan et al., 2012 and Newton and Roeselers, 2012) also the identification of unknown strain through comparing its sequence against databases in the Genbank. So, 16S rRNA gene sequencing has become one of the indispensable pillars in the polyphasic approach to bacterial classification and novel bacteria discovery (Woo et al., 2008) as in case of *Chryseobacterium* genus, the topic of this study.

The 16S rRNA sequence analysis succeeded in identification of the 2 sequenced isolates via BLAST search tool over the GenBank database as *Chryseobacterium jip* due to the 100% identity between the isolates of this study and *Chryseobacterium jip* 108/83 on the Genbank, representing 1.3 % of the examined 150 cow milk samples. Many authors could isolate and identify *Chryseobacterium* spp. from raw milk samples using the same method as Hantsis-Zacharov and Halpern (2007), Hantsis-Zacharov et al., (2008 a & b), Quigley et al., (2013), Boubendir et al., 2016, Yuan et al., 2017 and Porcellato et al., (2018). Unlike the results of the current study Giannino et al., (2009), Coton et al., (2012), Schirmer et al., (2013) and Aldrete-Tapia et al., (2014) could isolate *Chryseobacterium* spp. from cheese, while Jooste (1985) isolated *Chryseobacterium* spp. from butter.

Table 1: Incidence of the examined raw cow milk samples that showed *Chryseobacterium* spp. colonies:

Samples	No. of samples	Yellow color, Gram -ve, Oxidase & Catalase +ve colonies		Detection of flexirubin pigment by KOH (20%)			
				KOH +ve		KOH -ve	
		No.	%	No.	%	No.	%
Cow raw milk	150	10	6.7	2	20	8	80
Kariesh cheese	25	--	--	--	--	--	--
Domiatti cheese	25	--	--	--	--	--	--
Butter	25	--	--	--	--	--	--
Total	225	10	4.4	2	0.9	8	3.5

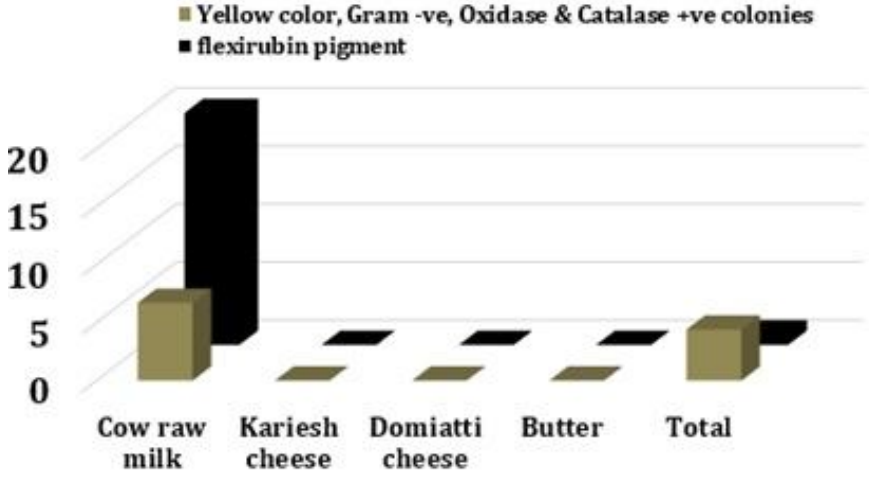

Fig. 1. Incidence of the examined samples that potentially contaminated with ***Chryseobacterium spp.***

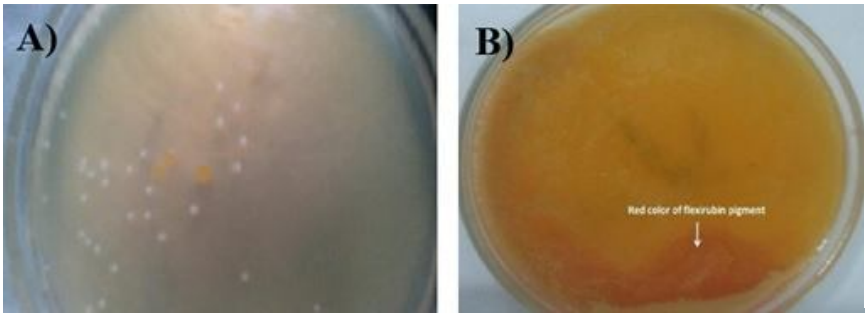

Fig. 2(A). Standard Plate Count agar showing yellow colonies. **Fig. 2(B).** Production of flexirubin pigment by flooding plates with KOH (20%).

In order to determine its exact taxonomic position of the obtained strains, a phylogenetic analysis carried out through construction of phylogenetic tree on the basis of the neighbor-joining method (Fig. 3), whereas the currently obtained isolates showed identity percentage ranging from 100%- 96% with respect to the other *Chryseobacterium* spp. species selected from the Genbank to construct the phylogenetic tree analysis. By referring to the source from which *Chryseobacterium* spp. that constructed the tree, it was found that the constructed phylogenetic tree not only determined the taxonomic position of the obtained *Chryseobacterium* spp., but also outlined the potential contamination sources or origin; and this can lead to new recommendations for public health policy in case of pathogens. The phylogenetic tree referred *Chryseobacteria* as an environmental flora (found in soil, water, milk, and food stuffs) rather than human flora, so, great attention should be paid for that organism to avoid possible public health hazards, for example, *C. indologenes* is known to be a lethal opportunistic pathogen, with inherent antimicrobial resistances (Lin et al., 2010).

By studying the phenotypic features of the confirmed *C. jip* in this study that were registered on the Genbank under proposed name *Chryseobacterium* Egyam strain AM1 (Accession No. MK059947) and *Chryseobacterium* Egyam strain AM2 (Accession No. MK059948), it was found that the isolated strains found to grow at 5ºC, 25 ºC and 37ºC while no growth noticed at 42ºC. The results of API 20 NE system declared that the both obtained strains were similar in their reaction to hydrolysis of gelatin and esculin, indole production, arginine hydrolysis, urease, ß-galactosidase, assimilation to mannitol, potassium gluconate, capric, adlpic, trisodium citrate and phenylacetic acid (Table 2). On the other hand, both isolates of *Chryseobacterium* exhibited dissimilarities in their biochemical reaction as shown in Table 2. As in Reduction of nitrates to nitrites, Assimilation of glucose, mannose, n-acetyl- glucosamine, maltose and malate tests, negative reactions were displayed by *Chryseobacterium* Egyam strain AM1 (MK059947) which was on contrary to *Chryseobacterium* Egyam strain AM2 (MK059948) that showed positive reaction. In opposite to the above-mentioned tests, *Chryseobacterium* Egyam strain AM1 (MK059947) assimilated arabinose, while *Chryseobacterium* Egyam strain AM2 (MK059948) couldn't.

Chryseobacterium spp. produce proteolytic and lipolytic enzymes; these enzymes make the presence of this genus in raw milk of an economical issue, not only for the reason that it causes off-odours and off-flavours like bitterness as well as gelation of milk, and spoilage in butter (Jooste et al., 1986; Harwalker et al., 1993; Huis in't Veld, 1996 and Tondo et al., 2004), but also because the proteolytic enzymes of a *Chryseobacterium* spp. have been shown to be resistant to pasteurization (Venter et al., 1999; Datta and Deeth 2003 and Yuan et al., 2018) constituting an

obstacle as a spoilage factor in pasteurized dairy products. In this study as shown in Table 2, species of *Chryseobacterium* showed more proteolytic than lipolytic activity based up on the results of API 20 tests where both *Chryseobacterium* Egyam strain AM1 (MK059947) and *Chryseobacterium* Egyam strain AM2 (MK059948) were positive for protease production through gelatin hydrolysis that is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin. This process takes place in two sequential reactions. In the first reaction, gelatinases degrade gelatin to polypeptides. Then, the polypeptides are further converted into amino acids (Pradhan, 2013). Additionally, both strains were expected to be negative for lipolytic activity through negative assimilation of capric and adlpic fatty acids. The results of our study suggest that it has high proteolytic but weak lipolytic activity.

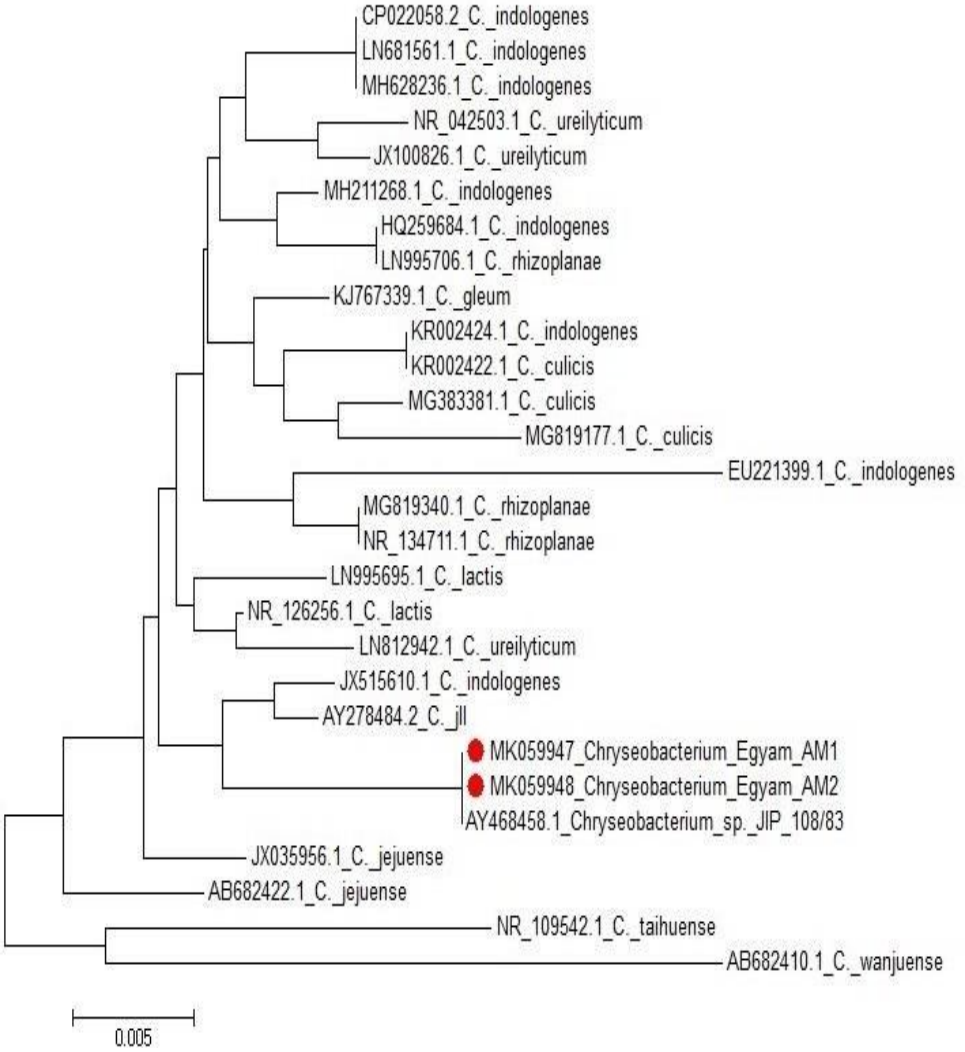

Fig. 3. Neighbor-joining tree showing the 16S rRNA gene phylogenetic identity relationships of the strains isolated from cow milk (MK 059947 and MK059948) and phylogenetically related reference strains on the GenBank.

Table 2: Phenotypic characteristics of the isolated strains:

Characters	*Chryseobacterium* Egyam AM1 (MK059947)	*Chryseobacterium* Egyam AM2 (MK059948)
Growth at different temperatures		
Growth at 5 ºC	+ve	+ve
Growth at 25 ºC	+ve	+ve
Growth at 37 ºC	+ve	+ve
Growth at 42 ºC	-ve	-ve
Flexirubin pigment production	+ve (strong)	+ve (strong)
API 20 NE		
Reactions/Enzymes		
Reduction of nitrates to nitrites	-ve	+ve
Reduction of nitrates to nitrogen	+ve	+ve
Indole production (tryptophan)	-ve	-ve
Fermentation (glucose)	-ve	-ve
Arginine dihydrolase	-ve	-ve
Urease	-ve	-ve
Hydrolysis (B-glucosidase)(Esculin)	+ve	+ve
Hydrolysis (protease)(Gelatin)	+ve	+ve
B-galactosidase (para-nitrophenyBd- Galactopyranosidase)	-ve	-ve
Assimilation (glucose)	-ve	+ve
Assimilation (arabinose)	+ve	-ve
Assimilation (mannose)	-ve	+ve
Assimilation (mannitol)	-ve	-ve
Assimilation (n-acetyl-glucosamine)	-ve	+ve
Assimilation (maltose)	-ve	+ve
Assimilation (potassium gluconate)	-ve	-ve
Assimilation (capric)	-ve	-ve
Assimilation (adlpic)	-ve	-ve
Assimilation (mala te)	-ve	+ve
Assimilation (trisodium citrate)	-ve	-ve
Assimilation(phenylacetic acid)	-ve	-ve

The isolated *Chryseobacterium* spp. exhibited close matching to the sequenced *Chryseobacterium jip* 108/83 that isolated by Bernardet et al. (2005) which showed on API 20 gallery negative fermentation of glucose, negative production of arginine dihydrolase and negative assimilation of malate and capric.

The ability of some species in this genus to grow at 5°C, in the presence of NaCl (0-5%) and over a wide PH range (Bekker, 2011) qualifies them to live in diverse types of food and environments; this ability is not constituting a spoilage problem only, but it makes these species is a matter of public health concern. Species of the genus *Chryseobacterium* are

emerging health care associated pathogens which mean, it is one of pathogens whose incidence has increased in the past 20 years and could increase soon, often colonizing the hospital environment.

Exploring and studying *Chryseobacterium* as a new genus introduced two important aspects of this genera; one is negative, while the other is positive. The negative aspect is outlined in its economic and public health importance, while the functionality of its bioactive compounds symbolizes its positive aspect. Recent studies of the genus *Chryseobacterium* have documented, the significance of its bioactive compounds as a bio control agent, antioxidant (Kim et al., 2012).

Conclusion

Chryseobacterium spp. could be isolated from raw milk samples only. Conventional method was not enough to confirm the isolates' identity, while 16S rRNA sequencing was pivotal in confirmation and identification of them on both genus and species level. The obtained isolates were identified as *Chryseobacterium jip*. Because of the spoilage and pathogenic character of this recently identified genus, regulatory standards for food manufacturers need to be improved through effective environmental monitoring programs, good manufacturing practice guidelines, and procedures and hazard analysis and critical control point (HACCP) systems to control the risk of microbiological contamination.

Acknowledgment

We are sincerely grateful for the help provided by all staff members of the Department of Food Hygiene, Faculty of Veterinary Medicine, South Valley University, Egypt.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abdalhamid B, Elhadi N, Alsamman K and Aljindan R (2016). *Chryseobacterium gleum* pneumonia in an infant with nephrotic syndrome. ID Cases.V. 5: 34–36.

Ahmad WA, Ahmed WY, Zakaria ZA and Yusof NZ (2012). Application of bacterial pigments as colorant. The Malaysian of perspective. Springer. DOI. Heidelberg Dordrecht. London. New York.

Aldrete-Tapia A, Escobar-Ramirez MC, Tamplin ML, Hernandez- Iturriaga M (2014). High-throughput sequencing of microbial communities in Poro cheese, an artisanal Mexican cheese. Food Microbiology, .44: 136-141.

Altschul SF, Gish W, Miller W, Myers EW and Lipmanl DJ (1990). Basic Local Alignment Search Tool. J. Mol. Biol., 215: 403-410.

Bauwens M, and J De Ley (1981). Improvements in the taxonomy of *Flavobacterium* by DNA:rRNA hybridization In: H. Reichenbach and O. B. Weeks (Eds.) The *Flavobacterium-Cytophaga* Group. Verlag Chemie. Weinheim, Germany. Gesellschaft für BiotechnologischeForschung Monograph Series, No. 5:27–31.

Bernardet JF, Nakagawa Y and Holmes B (2002). Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int. J. Syst. Evol. Microbiol., 52: 1049-1070.

Bernardet J-F, Vancanneyt M, Matte-Tailliez M, Grisez L, Tailliez P, Bizet C, Nowakowski M, Kerouault B, Swings J (2005). Polyphasic study of Chryseobacterium strains isolated from diseased aquatic animals. Syst. Appl. Microbiol. 28(7): 640-60.

Bekker A (2011): Growth and Spoilage Characteristics of *Chryseobacterium* species in milk. M.Sc.Thesis. Faculty of natural and agricultural Sciences. University of the free state

Bhalla MG, Gupta CS, Sarao MS and Kalra LD (2018). *Chreobacterium indologenes*: Case Report of an Emerging pathogene. IP: 193.227.49.2.

Boubendir A, Serrazanetti DI, Hamidechi MA, Vannini L, Guerzoni ME (2016). Changes in bacterial populations in refrigerated raw milk collected from a semi-arid area of Algeria. Annals of Microbiology, 66(2): 777-783.

Bloch KC, Nadarajah R, and Jacobs R (1997). *Chryseobacterium meningosepticum* an emerging pathogen among immunocompromised adults. Report of 6 cases and literature review. Medicine (Baltimore), 76: 30–41.

Booth SJ (2014). *Chryseobacterium* Related Genera Infections. Nebraska Medical Center, Omaha, NE, USA. https://doi.org/10.1016/B978-0-12-801238-3.04922-9.

Brkic´ DV, Zlopaša O, Bedenic´ B and Plecko V (2015). *Chryseobacterium gleum* infection in patient with extreme malnutrition and hepatic lesion-case report.Signa Vitae.V.10:P.50-52.

Chaudhari PN, Wani KS, Chaudhari BL and Chincholkar SB (2009). Characteristics of sulfobacinAfromasoilisolate *Chryseobacterium gleum*. Appl.Biochem. Biotechnol, 158: 231–241.

Clarridge JE 3rd (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev. 17: 840–862.

Conlan S, Kong HH and Segre JA (2012). Species-level analysis of DNA sequence data from the NIH Human Microbiome Project. PMID: 23071716.

Coton M, Delbés-paus C, Irliger F, Desmasures N, Lefleche A, Stahl V, Montel MC and Coton E (2012). Diversity and assessment of potential risk factors of Gram-negative isolates associated with French cheeses. Food microbial., 29: 88-98.

Datta N and Deeth HC (2003). Diagnosing the cause of proteolysis in UHT milk Lebensmittel- Wissenschaft und-Technologie., 36: 173–182.

De Carvalho Filho EB, Marson F, Levy CE (2017). Challenges in the identification of *Chryseobacterium indologenes* and *Elizabethkingia meningoseptica* in cases of nosocomial infections and patients with cystic fibrosis.New Microbes New Infect., 13: 20-27.

Fautz E & Reichenbach H (1980). A simple test for flexirubin-type pigments. FEMS Microbiol Lett, 8: 87–91.

García-López ML, Santos J-A and Otero A (1999)> Flavobacterium. In: R.K.I Robinson, R.K.I. (Ed.), Encyclopedia of Food

Microbiology. Academic Press, San Diego, p. 820-824.

Garg S, Appannanavar SB, Mohan B, Taneja N (2015). Pyonephrosis due to *Chryseobacterium gleum*: a first case report. Indian J. Med. Microbiol., 33: 311-313.

Giannino ML, Marzotto M, Dellaglio F and Feligini M (2009). Study of microbial diversity in raw milk and fresh curd used for Fontina cheese production by culture-independent methods. International Journal of Food Microbiology, 130(3): 188-195.

González-Castro A, Alsasua A, Penasco Y, Rodríguez JC and Duerto J (2017). Tracheo-bronchitis and pneumonia associated with mechanical ventilation by *Chryseobacterium indologenes*. Rev Esp Anestesiol Reanim., 64(5): 294-298.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis. Program for Windows 95/98/NT. Nucl. Acids. Symp. Ser., 41: 95-98.

Hantsis-Zacharov E and Halpern M (2007). *Chryseobacterium haifence* sp. nov., a psychrotolerant bacterium isolated from raw milk. International journal of systematic and evolutionary microbiology, 57: 2344-2348.

Hantsis-Zacharov E, Senderovich Y & Halpern M (2008a): *Chryseobacterium bovis* sp. Nov., Isolated from raw cow's milk. International Journal of systematic and evolutionary microbiology, 58: 1024-1028.

Hantsis-Zacharov E, Shake´ dT, Senderovich Y and Halpern M. (2008b). *Chryseobacterium* oranimense sp. nov., a psychrotolerant, proteolytic and lipolytic bacterium isolated from raw cow's milk. International Journal of Systematic and Evolutionary Microbiology, 58: 2635–2639.

Harwalker VR, Cholette H, McKellar RC, and Emmons DB (1993). Relation between proteolysis and astringent off-flavor milk. J. Dairy Sci. 76: 2521–2527.

Huisint Veld JH (1996). Microbial and biochemical spoilage of foods: an overview. Int J Food Microbiol. 33(1):1-18.

Imataki O and Uemura M (2016). *Chryseobacterium indologenes*, a possible emergent organism resistant to carbapenem antimicrobials after stem cell transplantation. Clinical Case Reports, 5(1): 22–25.

ISO "International Organization for Standardization" 8261. (2001): General guidance for preparation of test samples, initial suspentions and decimal dilutions for microbiological examination of milk and milk products.

Jain V, Amrin N, Hussain FA, Siddiqui T, Sahu C, Ghar M and Prasad KN (2017). Simultaneous isolation of *Chryseobacterium gleum* from bloodstream and respiratory tract: first case report from India. J.M.M. Case Reports:4.

Jooste PJ (1985). The taxonomy and significance of flavobacterium cytophaga strains from dairy sources. PhD Thesis, university of the orange free state, South Africa.

Jooste PJ and Hugo CJ (1999). The taxonomy, ecology and cultivation of bacterial genera belonging to the family *Flavobacteriaceae*. International Journal of Food Microbiology, 53: 81-94.

Jooste PJ, Britz TJ, Lategan PM (1986). The prevalence and significance of flavobacterium strains in commercial salted butter. Milchwissenschaft 41, 69-73.

Kim HS, Sang MK, Jung HW, Jeun YC, Myung IS and Kim KD (2012). Identification and characterization of *Chryseobacterium wanjuense* strain KJ9C8 as a biocontrol agent of Phytophthora blight of pepper Crop Protect., 32: 129–137.

Kim KK, Bae HS, Schumann P and Lee S T (2005). *Chryseobacterium daecheongense* sp. Nov., Isolated from fresh water lake sediment, Int. J. Syst. Evol. Microbiol., 55: 133-138.

Krebs J, Sampsell D, McDonald C, and Newman J (2012). Purification and Molecular Structure Determination of Flexirubins from *Chryseobacteria*. Poster presented at the 2012 Undergraduate Research Symposium in the Chemical and Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.

Krebs J, Gale A and Newman J (2013). Purification of Flexirubin Pigments from *Chryseobacterium*. Poster, undergraduate scholarship program. Lycoming College.

Lampert LM (1975). Modern Dairy Products. 3rd Ed., Chemical Pub. Co., Inc., New York.

Land G, Mcginris MR, Staneck J and Gaston A (1991). Aerobic pathogenic actinomycetales . In: Manual of clinical microbiology. 5th ED. 340-360.

Lagacé L, Pitre M, Jacques M, and Roy D (2004). Identification of the Bacterial Community of Maple Sap by Using Amplified Ribosomal DNA (rDNA) Restriction Analysis and rDNA Sequencing. Applied and environmental microbiology. 2052–2060.

Lin Y-T, Jeng Y-Y, Lin M-L, Yu K-W, Wang F-D, Liu C-Y (2010). Clinical and microbiological characteristics of *Chryseobacterium* indologenes bacteremia. J. Microbiol. Immunol. Infect., 43: 498–505.

Lin J-N, Teng S-H, Lai C-H, Yang C-H, Huang Y-H, Lin H-F, Lin H-H and Geoffrey A (2018). Comparison of the Vitek MS and Bruker Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of *Chryseobacterium* Isolates from Clinical Specimens and Report of Uncommon *Chryseobacterium* Infections in Humans. American Society for Microbiology, 56 (11): 25.

Lo HH and Chang SM (2014). Identification, characterization and biofilm formation of clinical *Chryseobacterium gleum* isolates. Diagn Microbiol Infect. Dis., 79: 298–302.

Mudarris M, Austin B, Segers P, Vancanneyt M, Hoste B, Bernardet JF (1994). Flavobacterium scophthalmum sp. nov., a pathogen of turbot (Scophthalmus maximus L.)". International Journal of Systematic Bacteriology, 44(3): 447–453.

Newton IL and Roeselers G (2012). The effect of training set on the

classification of honeybee gut microbiota using the Naive Bayesian Classifier. BMC Microbiol., 12: 221. PMID: 23013113.

Omar A, Camara M, Fall S, Ngom-Cisse S, Fall B and Ba-Diallo A (2014). *Chryseobacterium indologenes* in a woman with acute leukemia in Senegal: a case report. J. Med. Case Rep., 8: 138.

Porcellato D, Aspholm M, Skeie SB, Monshaugen M, Brendehaug J and Mellegard H (2018). Microbial diversity of consumption milk during processing and storage. International Journal of Food Microbiology, 266: 21–30.

Pradhan P (2013). Gelatin hydrolysis test: Principle, Procedure, Interpretation and preparation of nutrient gelatin medium. Microbiology and infectious diseases.

Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF & Cotter PD (2011): Molecular approaches to analyzing the microbial composition of raw milk and raw milk cheese. Int. J. Food Microbiol., 150: 81–94.

Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF and Cotter PD (2013). The complex microbiota of raw milk. FEMS Microbiol Rev., 37: 664–698.

Rawat A, Vignesh P, Sharma A, Shandilya JK, Sharma M (2017). Infection profile in chronic granulomatous disease:a 23-year experience from a tertiary care center in North India. J. Clin. Immunol., 37: 319–328.

Rajendran P, Muthusamy S, Balaji VK, Rakesh GJ and Easow JM (2016). Urinary tract infection due to *Chryseobacterium gleum* pathogen. Indian J. Pathol. Microbiol., 59: 551–553.

Saleem HG, Seers CA, Sabri AN and Reynolds EC (2016). Dental plaque bacteria with reduced susceptibility to chlorhexidine are multidrug resistant. BMC.Microbiol. 16: 214.

Schirmer BCT, Heir E, Møretrø T, Skaar I and Langsrud S (2013). Microbial background flora in small-scale cheese production facilities does not inhibit growth and surface attachment of *Listeria monocytogenes*. J. Dairy Sci., 96: 6161–6171.

Segers PW, Mannheim M, Vancanneyt K, De Brandt K-H, Hinz K, Kersters and Vandamme P (1993). *Riemerella anatipestifer* gen. nov., the causative agent of septicemia anserum exsudativa, and its phylogenetic affiliation within the *Flavobacterium-Cytophaga* rRNA homology group. Int. J. Syst. Bacteriol. 43:768–776.

Thompson JD, Higgins DG and Gibson TJ (1994). Nucleic Acids Research, 22(22): 4673-4680.

Tondo EC, Lakus FR, Oliveira FA and Brandelli A (2004). Identification of heat stable protease of Klebsiella oxytoca isolated from raw milk. Letters in Applied Microbiology, 38: 146-150.

Vacheyrou M, Normand AC and Guyot P. (2011). Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms. Int. J. Food Microbiol., 146(3): 253-262.

Vancanneyt MP, Segers U, Torck B, Hoste J-F, Bernardet P, Vandamme and Kersters K (1996). Reclassification of *Flavobacterium odoratum* (Stutzer 1929) strains to a new genus, *Myroides*, as *Myroides*

odoratus comb. nov. and *Myroides odoratimimus* sp. nov. Int. J. Syst. Bacteriol., 46: 926-932.

Vandamme P, Bernardet J-F, Segers K, Kersters K & Holmes B (1994). Nprespectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., Bergeyella gen. nov., and Empedobacter nom. Rev. International Journal of systematic bacteriology, 44: 827-831.

Venile ChK, Zakaria ZA, Usha R and Ahmad WA (2014). Isolation and characterization of flexirubin type pigment from *Chryseobacterium* sp. UTH3T Biocatalysis and Agricultural Biotechnology, 3: 103–107.

Venile ChK, Zakaria ZA and Ahmad WA (2015). Optimization of culture conditions for flexirubin production by *Chryseobacterium artocarpi* CECT 8497 using response surface methodology. Biochemica polonica, 62 (2): 185-190.

Venter H, Osthoff G, and Litthauer D (1999). Purification and characterization of a metalloprotease from *Chryseobacterium indologenes* Ix9a and determination of the amino acid specificity with electrospray mass spectrometry. Protein Expression and Purification. 15 (2): 282 295.

Virok DP, Ábrók M, Szel B, Tajti Z, Mader K (2014). *Chryseobacterium gleum* – a novel bacterium species detected in neonatal respiratory tract infections. J. Matern Fetal Neonatal., 27: 1926-1929.

Vithanage NR, Dissanayake M, Bolge G, Palombo EA, Yeager TR and Datta N (2016). Biodiversity of culturable psychrotrophic microbiota in raw milk attributable to refrigeration conditions, seasonality and their spoilage potential. Int. Dairy J., 57: 80–90.

Woo PC, Lau SK, Teng JL, Tse H, Yuen KY (2008). Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect., 14: 908–934.

Xin L, Meng Z, Zhang L, Cui Y, Han X, Yi H (2017). The diversity and proteolytic properties of psychrotrophic bacteria in raw cows' milk from North China. International Dairy Journal, 66: 34-41.

Yuan L, Sadiq FA, Liu T, Flint S, Chen J, Yang H, Gu J, Zhang G, He G, (2017). Psychotrophic bacterial populations in Chinese raw dairy milk. LWT - Food Science and Technology, 84: 409-418.

Yuan L, Sadiq FA, Liu T-J, Li Y, Gu J-S, Yang H, He G (2018). Spoilage potential of psychrotrophic bacteria isolated from raw milk and thermos-stability of their enzymes. J. Zhejiang Univ-Sc 630 (Biomed & Biotechnol), 19(8): 630-642.