REVIEW

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Small Colony Variants of *Staphylococcus aureus* – *review*

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ABSTRACT. Bacterial variants of *Staphylococcus aureus* called small colony variants (SCVs) originate by mutations in metabolic genes, resulting in emergence of auxotrophic bacterial subpopulations. These variants are not particularly virulent but are able to persist viable inside host cells. SCVs show their characteristic auxotrophic growth deficiency and depressed α-cytotoxin activity. Environmental pressure such as antibiotics, select for isogenic SCV cells that are frequently found coexisting with their parent wild-type strains in a mixed bacterial culture. SCV strains often grow on blood agar as non-pigmented or pinpoint pigmented colonies and their key biochemical tests are often non-reactive. Their altered metabolism or auxotrophism can result in long generation time and thus SCV phenotype, more often than not SCV can be overgrown by their wild-type counterparts and other competitive respiratory flora. This could affect laboratory detection. Thus, molecular methods, such as 16S rRNA partial sequencing or amplification of species-specific DNA targets (*e.g.* coagulase, nuclease) directly from clinical material or isolated bacterial colonies, become the method of choice. Patients at risk of infection by *S. aureus* SCVs include cystic fibrosis patients (CF), patients with skin and foreign-body related infections and osteomyelitis, as they suffer from chronic staphylococcal infections and are subject to long-term antibiotic therapy. Molecular evidence of SCV development has not been found except for some random mutations of the thymidylate synthase gene (*thyA*) described in SCV *S. aureus* strains of CF patients. These variants are able to bypass the antibiotic effect of folic acid antagonists such as sulfonamides and trimethoprim. Resistance to gentamicin and aminoglycosides in the hemin or menadione auxotrophic SCVs was hypothesized as being due to decreased influx of the drugs into cells as a result of decreased ATP production and decreased electrochemical gradient on cell membranes.

Abbreviations

CF	cystic fibrosis (a genetic disorder)
CFTR (gene)	gene for cystic fibrosis transmembrane conductase regulator
MLST	multiple-locus sequence typing
MRSA	methicillin-resistant *S. aureus*
PFGE	pulsed field gel electrophoresis
SCVs	small colony variants
spa (typing)	single-locus analysis of structural gene (*spa*) for protein A of *S. aureus*
thyA	thymidylate synthase A gene
VNTR	multiple-locus variable tandem repeat analysis

CONTENTS

1 Definition and history of bacterial small colony variants 548
2 Definition and history of small colony variants of *S. aureus* 549
3 Risk patients 549
4 Auxotrophy and origin 550
5 Cultivation 551
6 Stability 552
7 Phenotypic identification 553
8 Genotypic identification 553
9 Susceptibility to antibiotics 554
10 Intracellular persistence 554
References 556

1 DEFINITION AND HISTORY OF BACTERIAL SMALL COLONY VARIANTS

Small colony variants (SCVs) constitute a slow-growing auxotrophic subpopulation of bacteria with distinctive phenotypic and pathogenic traits (Proctor *et al.* 2006). SCVs, also known as dwarf colony variants, were described first in *Salmonella* Typhi in 1910 by Jacobsen (Stokes and Bayne 1958). A significant number of clinically relevant bacteria forming small colony variants have been described, *e.g.*, from *E. coli*, *Shigella*, *Vibrio*, *Bacillus*, *Corynebacterium* (Borderon and Horodniceanu 1978), *Serratia marcescens* (Koh *et al*. 2007), *Neisseria gonorrhoeae* (Morton and Shoemaker 1945), *Pseudomonas aeruginosa* (Häussler *et al*. 1999), *Burkholderia cepacia* (Häussler *et al*. 2003) and *Stenotrophomonas maltophilia* (Anderson *et al.* 2007).

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Often occurring in periods of chronic bacterial infections, selection pressure of the environmental factors (also including antibiotics) causes spontaneous mutations in housekeeping genes which diversify bacterial populations (Oliver *et al*. 2000; Besier *et al*. 2007, 2008). These mutations often lead to SCVs, which are declared as deficient in electron transport with increased resistance to aminoglycosides (Proctor *et al.* 1998). The increased fitness of SCV bacteria afford them a higher chance to resist the host defenses because of, *e.g*., serum resistance (Häussler *et al*. 2003*a*), hyper-adherence to the host cells (Malone *et al.* 2010) or intracellular persistence (Proctor *et al*. 1995). The following chapters deal with information characterizing only SCV *Staphylococcus aureus*.

2 DEFINITION AND HISTORY OF SMALL COLONY VARIANTS OF *S. aureus*

SCVs have been found in different staphylococcal species such as *S. lugdunensis* (Seifert 2005), *S. capitis* and *S. epidermidis* (von Eiff *et al.* 1999). The information describing SCVs of *S. aureus* was published already in the thirties, forties (*see* Proctor *et al.* 1998) and fifties (Sherris 1952; Goudie and Goudie 1955; Thomas and Cowlard 1955). Because of only sporadic case reports and a limited number of methods to analyze auxotrophy, clinical microbiologists were interested in this topic only marginally (Goudie and Goudie 1955; Thomas and Cowlard 1955). However, since 1994 when Proctor and his colleagues characterized biological and pathogenic traits of *S. aureus* SCV strains on molecular level, a new era started (Proctor *et al.* 1995). Currently, increasing evidence is showing that *S. aureus*, especially SCVs, can persist intracellularly in the host for decades (Proctor *et al.* 1998). This fact changed the concept of *S. aureus* as an extracellular pathogen to a pathogen with an intracellular part of its life cycle (Garzoni and Kelley 2009; Sendi and Proctor 2009).

3 RISK PATIENTS

A few categories of patients who undergo long-term antibiotic therapy are at risk to acquire the infection. Special attention should be paid to patients with CF, more than 70 % of which were colonized or infected with *S. aureus* or SCVs during their lifetime (Kahl *et al.* 1998, 2003*a*). CF is a genetic disorder caused by a mutation in CFTR gene affecting a transmembrane chloride channel resulting in, among others, abnormal respiratory secretions increasing the probability of infections. *S. aureus* together with *Haemophilus influenzae* are the first bacterial agents infecting the lower respiratory tract of CF patients. Some patients who initially harbored both normal and SCV strains subsequently lost the normal strain, while SCVs persisted for an extended period (von Eiff *et al.* 2008) (Figs 1, 2 and 3). Probably analogously to *P. aeruginosa* infection,

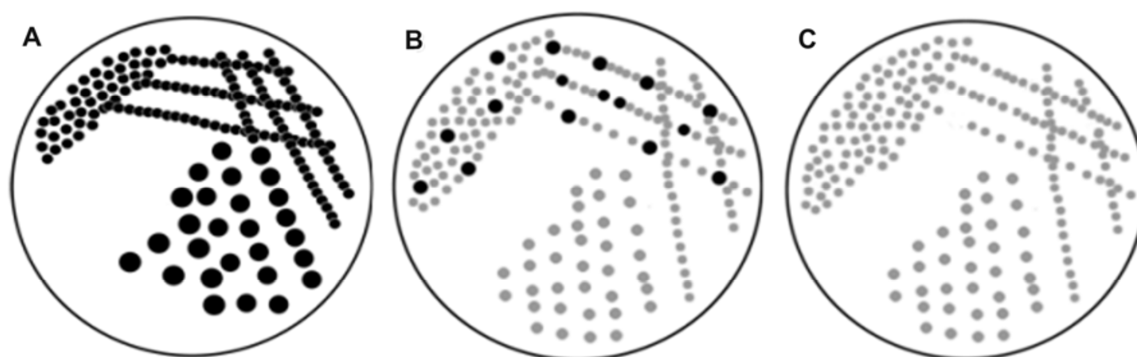

Fig. 1. Diagram of bacterial culture from acute (**A**) and chronic (**B**,**C**) staphylococcal infection of CF patient. Changed proportion of parent wild-type and SCV *S. aureus* cells could be detected on blood agar because their phenotypes differ (schematically: wild-type colonies and SCV colonies in *black* and *grey* color, respectively) (diagram – authors).

polymorphonuclear cells migrating into inflamed lung tissue and cytokines released are responsible for the activation of immunopathological processes in the affected organ and deterioration of lung function (Hoiby *et al.* 2001). Exposure of lung tissue to long-term irritation and antibiotic treatment causes the patients to be at a higher risk of infection with chronic bacterial pathogens, *e.g*., *P. aeruginosa* and *B. cepacia*, and the prognosis of the patients may deteriorate (Hoiby *et al*. 2001; Lyczak 2002).

Other categories of chronic patients susceptible to SCV *S. aureus* infections, usually receiving aminoglycosides, suffer from skin disorders (von Eiff *et al.* 2001), osteomyelitis (von Eiff *et al.* 1997*a*; Proctor *et al.* 1998) or foreign-body related infections (von Eiff and Becker 2007).

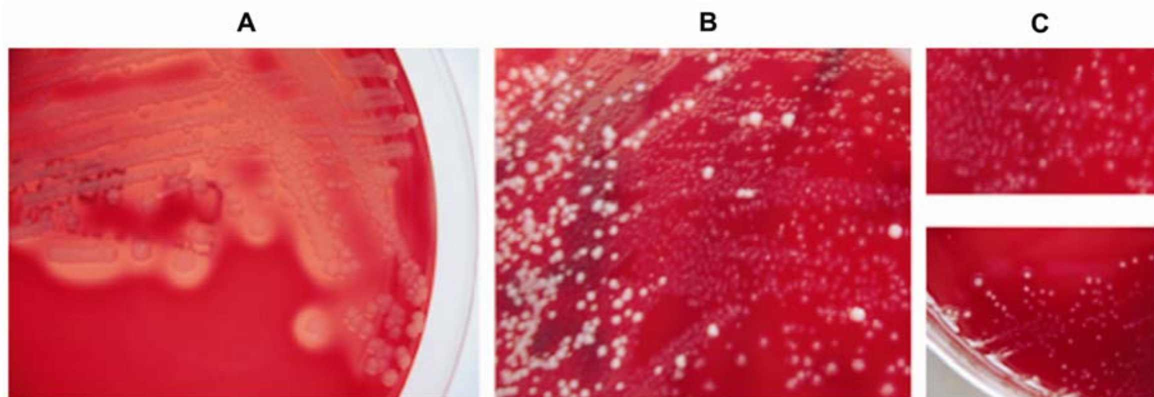

Fig. 2. Bacterial culture from acute (**A**) and chronic (**B**,**C**) staphylococcal infection of CF patient. Hemolytic and/or pigmented colonies are typical for the acute (**A**), pigmented and nonpigmented small grey (**B**,**C** *top*) or "fried eggs" colonies (**C** *bottom*) for the chronic phase (photo – authors).

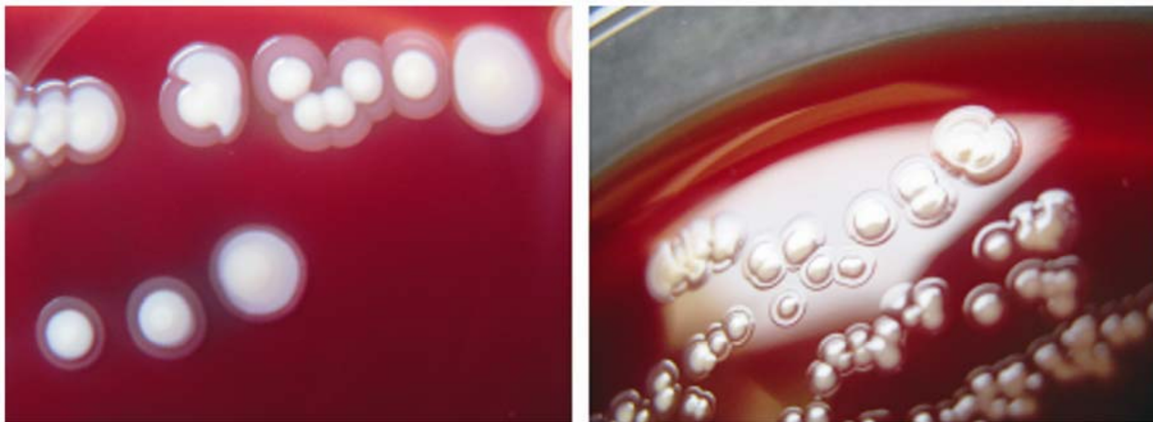

Fig. 3. Detail of "fried eggs" colonies of SCV *S. aureus* strains growing on blood agar (photo – authors).

4 AUXOTROPHY AND ORIGIN

Certain strains of *S. aureus* can *in vitro* (Massey *et al.* 2001) and *in vivo* (Goudie and Goudie 1955; Proctor *et al.* 1998; Kahl *et al*. 1998; von Eiff *et al.* 1997*b*, 2001) produce a subpopulation which is phenotypically very different from the parent strain. This subpopulation originates by spontaneous mutation, or the mutation is induced by antibiotics (Massey *et al.* 2001; Lånnergard *et al.* 2008). If the selective pressure of antibiotics lasts for long, the number of changed cells in the bacterial population increases relative to their parent wild-type cells. The SCV cells have a longer generation time, so they grow slowly. This leads to colonies which are approximately one-tenth the size of "normal" *S. aureus*, hence the name "small colony variants". The features of these SCVs include both decreased pigmentation and hemolysis. They generally show evidence of an unstable colony phenotype.

The biochemical basis of this phenotypic abnormality is a single or multiple auxotrophism caused by mutations of genes involved in the biosynthesis of thiamine, menadione, hemin or thymidine (Bentley and Meganathan 1982; Lånnergard *et al*. 2008; Chatterjee *et al*. 2008; Besier *et al*. 2007; Schaaff *et al*. 2003; Proctor *et al.* 2006). Any of these defects results in reduced function of certain metabolic pathways, such as the electron transport chain *per se*, or the tricarboxylic-acid cycle, both resulting in a decreased production of ATP (Chatterjee *et al.* 2007; Kohler *et al*. 2003; Seggewiss *et al*. 2006; von Eiff *et al.* 2006).

Menadione, hemin and thiamine are required for biosynthesis of electron transport-chain components. Menadione is isoprenylated to form menaquinone, the acceptor of electrons from NADH/$FADH_2$ in the

electron transport chain. Hemin (von Eiff *et al.* 1997*b*) is required for the biosynthesis of cytochromes, which accept electrons from menaquinone and complete the electron transport chain. Thiamine is required for menadione synthesis (Bentley and Meganathan 1982), hence thiamine auxotrophs are also menadione auxotrophs. Interruption of electron transport results in a decreased electrochemical gradient and as a consequence reduced quantities of ATP. Large amounts of ATP are required for cell-wall biosynthesis, thus the slow growth leads to small colonies. The electron transport is also directly linked to the biosynthesis of carotenoid pigments, rendering the colonies non-pigmented. The limited hemolysis and slow coagulase reaction is in part related to decreased amino acid biosynthesis (Proctor *et al.* 1998). The supplementation of the lost key compounds to the growth medium revert the SCVs to normal size colonies.

Experiments with aminoglycoside-induced SCVs showed formation of hemin–auxotrophic SCV. These SCVs harbored two mutations; a deletion in *hemH* and a frame shift in *hemA*. Both genes are involved in the biosynthesis of hemin (Schaff *et al.* 2003). Also menadione-auxotrophic isogenic SCV and wild-type strains from three different patients with chronic osteomyelitis receiving systemic antibiotic therapy were analyzed (Lånnergard *et al*. 2008). Mutations or deletions in the *menB* gene involved in menadione biosynthesis and reduced susceptibility to gentamicin were detected in the SCV isogenic strains. In the wild-type revertants back mutations or secondary site compensatory mutations in the *menB* gene were detected. Reversion of hemolytic activity and MIC for gentamicin, however, would not necessarily be restored even if caused by the same original mutation in *menB* (Lånnergard *et al.* 2008). However, the actual genetic basis of SCVs recovered from clinical specimens is still largely unknown, with an exception of two recent studies showing that thymidine-auxotrophic SCVs from cystic fibrosis patients carry mutations in the thymidylate synthase gene (*thyA*) (Besier *et al*. 2007; Chatterjee *et al*. 2007, 2008) (Fig. 4). Thymidine-auxotrophic *S. aureus* SCVs show resistance to trimethoprim–sulfamethoxazole, which normally interferes with the tetrahydrofolic acid pathway. Thymidylate synthase (*thyA*) requires tetrahydrofolic acid as a cofactor to catalyze the last step of the thymidine biosynthesis pathway, which is responsible for the formation of dTMP. Thus, mutations in *thyA* provide a possible explanation for thymidine auxotrophy and thereby resistance to the above mentioned antibiotics, and the SCV phenotype. SCV strains complemented with a plasmid carrying intact *thyA* gene get their wild-type phenotype fully restored (Besier *et al*. 2007; Chatterjee *et al.* 2007, 2008).

Fig. 4. Thymidine auxotrophic strain. The auxotrophy is tested by placing a disk with thymidine onto a Mueller–Hinton agar streaked by culture of SCV *S. aureus* strains from a CF patient and cultivated up to 3 d. The developed colonies differ in size and pigmentation from parent strain (photo – authors).

Formation of SCV phenotype can also be influenced by differentially expressed non-coding RNAs (npcRNAs) in SCVs and wild-type strains of *S. aureus* (Abu-Quatouseh *et al*. 2010). The RNA molecules also known as central regulatory molecules are short molecules of RNA that are not translated into proteins but rather exert various regulatory functions encoded by RNA itself or in complexes with proteins. Except for different npcRNA expression pattern originally transcribed npcRNA genes (Sau-66) were also detected only in SCV phenotype (Abu-Quatouseh *et al*. 2010).

Most of the studies regarding metabolic pathways of SCVs were performed on genetically defined laboratory mutants (*e.g.,* JB1, IIb13, A378 I) because of their stable phenotype (Tuscher *et al.* 2010; Abu-Quatouseh *et al*. 2010).

5 CULTIVATION

SCVs could be detected on various types of enriched cultivation media in aerobic or microaerophilic atmosphere usually after 3 d (Fig. 5). Even though the colonies can be cultured in many different atmospheres, enlarged colonies of SCV staphylococci could be seen on blood agar plates cultivated in microaerophilic atmosphere. This led to the assumption of some dependence on CO_2 (Thomas and Cowlard 1955; Sherris 1952; Goudie and Goudie 1955; Kahl *et al.* 1998; Schneider *et al*. 2008). Dwarf nonhemolytic and nonpigmented colonies of SCVs are approximately ten times smaller than parent strain when grown on blood agar in aerobic atmosphere at 37 °C (Youmans and Delves 1942; Sherris 1952; Goudie and Goudie 1955; von Eiff *et al*. 2001; Kahl *et al*. 2003*a*). "Fried-egg" colonies with protrudent creamy colony center or pinpoint colonies are typical for many SCV strains (Kahl *et al*. 2003*a*).

Because of the biological properties of SCVs they can easily be overgrown, which constitutes problems in their cultivation. The overgrowth can be caused by commensal flora or wild-type staphylococci because of the long generation time of SCV *S. aureus*. This could be minimized, though, by dilution of clinical material before plating it onto the culture media (Tebbutt and Coleman 1978). Because of the limitation caused by the commensal flora, it was suggested to use differential culture media such as blood agar with 5 % of NaCl (Kahl *et al*. 1998) or chromogenic media (Kipp *et al.* 2005) to detect them. As a nutritionally rich

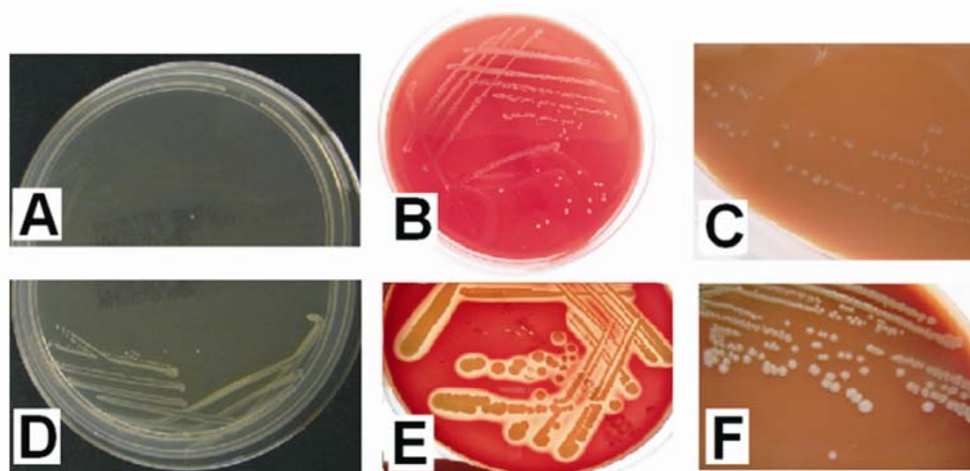

Fig. 5. Cultivation of SCV *S. aureus* (**A**–**C**) and *S. aureus* parent wild-type strain (**D**–**F**) on nutrient, blood and chocolate agar (from *left* to *right*) in aerobic atmosphere. SCV *S. aureus* strains of CF patients are not growing on nutrient agar because of their mutations in thymidylate synthase gene (*thyA*) and auxotrophy to thymidine (Besier *et al*. 2007) which is not included in the agar media (photo – authors).

undefined medium Schaedler agar is suitable to restore normal *S. aureus* phenotype (Kahl *et al*. 1998; Schneider *et al.* 2008) probably because it is a source of metabolic precursors for SCV population (*e.g.*, amino acids, thymidine, thiamine, vitamin K_1) (Melter, *unpublished results*). When the metabolism of SCV strains is changed by anaerobiosis or cool incubation (30 °C), they grow like typical staphylococcal colonies (Thomas and Cowlard 1955; Goudie and Goudie 1955). When grown in tubes with the caps tightly screwed, their growth become heavy like the normal *S. aureus* – no doubt due to the rise in CO_2 concentration derived from the organism's own metabolic processes (Goudie and Goudie 1955).

6 STABILITY

While a few stable SCV strains are known (*e.g.* JB1), the majority of colonies of SCV phenotype are unstable and revert to normal phenotype by compensatory mutations (Tuchscherr *et al*. 2010; Becker *et al.* 2006; Lånnergard *et al.* 2008). Stability of SCV phenotype was studied using different culture media, atmospheres and temperature as described *above*. SCVs commonly have condition-dependent, media-dependent and time-dependent phenotype (Youmans and Delves 1942; Kaplan and Dye 1976) (Figs 6 and 7).

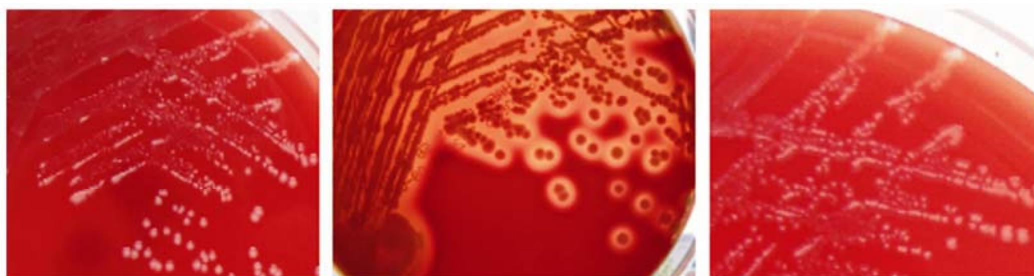

Fig. 6. Media-dependent stability. SCV *S. aureus* strain cultivated on blood agar (*left*) develops into the wild-type phenotype in culture on Schaedler agar in microaerophilic atmosphere (*center*) and develops again into the SCV phenotype after recultivation on blood agar (*right*). The reversion is not genetically determined.

The stability of SCV colonies was also studied by periodical subculture of the colonies for longer time under the same conditions and media. It was shown that some SCVs were stable *in vitro* for at least 40 passages (Goudie and Goudie 1955). The stability of SCV phenotype *in vivo* cultivated for years or decades from particular patients was also described by Proctor *et al.* (1998). On the other hand, when SCV colonies are heaped up on the medium, the aggregate is opaque and creamy and, after exposure to daylight at room temperature for a few hours, it develops the golden yellow pigment typical for *S. aureus* (Goudie and Goudie 1955) or even a pronounced pigment more orange than original culture (Youmans and Delves 1942) (Fig. 7). This change is probably due to compensatory mutations, as suggested by Lånnergard *et al.* (2008).

Fig. 7. Time-dependent stability; culture of SCV *S. aureus* cultivated for 24 h at 37 °C and then stored at room temperature in the light. If the inoculum is attained by a loop from a grown culture and stored in the light at room temperature, new wild-type colonies will grow until the next day (*yellow* streak). New wild-type colonies are also growing after a few days among SCV colonies (*cf.* Goudie and Goudie 1955) probably because of some compensatory mutations (*cf.* Lånnergard 2008). The reverted cells can probably use also products synthesized by the SCV strains (*e.g.*, carbon dioxide, enzymes) (Melter, *unpublished results*) (photo – authors).

7 PHENOTYPIC IDENTIFICATION

Due to colonial appearance of SCVs they could be misidentified as commensal corynebacteria or non-hemolytic streptococci. Gram-stained round shaped cells of SCV, even if not regular, contrast with the club shaped cells of corynebacteria. A crucial step in preliminary identification of SCVs from CF patients is a positive catalase test (even if delayed) which differentiates SCV staphylococci from catalase-negative group and the Viridans and γ-streptococci, which are a common part of respiratory tract flora. An oxidase-negative test also discriminates them promptly from the oxidase positive oropharyngeal neisseriae. Microscopy made of the culture can confirm Gram-positive staphylococci in clusters even if particular SCV strains have larger and more irregular cells similar to *Micrococcus* and related genera (Quie 1969). Electron microscopy reveals enlarged cocci with incomplete or multiple cross-links in their walls as a consequence of impaired cell separation (von Eiff *et al*. 2001; Kahl *et al*. 2003*b*). Altered metabolic activity of SCVs could likely influence the results of the biochemical tests. Biochemical reactions tested in clinical microbiology laboratory show the utilization of glucose and fructose but not mannitol. Thus, SCVs are often difficult to recognize as staphylococci also if biochemical assays have been applied, and they may thus be misidentified by routine identification approach (Proctor *et al*. 1995). Because of delayed production of coagulase, the test for detection of free coagulase should be read first after 3 d (Kahl *et al*. 1998; Proctor *et al*. 1998). Commercial systems for detection of bound coagulase combined with detection of protein A (*Pastorex Staph Plus*, *Biorad*) could be negative in a small portion of analyzed SCV strains. While in some cases the identification of staphylococcal SCVs by modern (semi)automatic systems may be successful, these approaches often fail to identify and distinguish this phenotype (*e.g.*, Api ID 32 Staph, VITEK; *bioMeriéux*, France) (Kahl *et al*. 1998; Sadowska *et al*. 2002; Becker *et al*. 2004).

8 GENOTYPIC IDENTIFICATION

Specific genes of *S. aureus* could be detected directly in clinical material or from pure bacterial culture by using commercial systems (Schneider *et al*. 2008). Experimentally introduced PCR reactions for detection of *nuc*, *coa* genes and 16S rRNA were also used (von Eiff *et al*. 2001; Becker *et al.* 2004; *National Food Institute* 2008). Another important useful tool to identify also SCV *S. aureus* phenotypes is a sequenced-based method comparing 5′ end of DNA for 16S rDNA of analyzed isolates with the database of type strains (Becker *et al*. 2004). Higher sensitivity of the amplification methods shows advantages over culture.

Genotypic methods except species identification also delineate clonality of the analyzed *S. aureus* strains. Colonization or infection of patients with parent and isogenic daughter SCV strains or various clones

of *S. aureus* could be determined. Also *in vivo* stability of SCV phenotype in the same patients could be confirmed by molecular methods (Kahl *et al*. 1998).

Various band-based or sequence-based molecular methods are used. The most frequent and useful method is macrorestriction analysis of chromosomal DNA by pulsed field gel electrophoresis (PFGE) (Kahl *et al*. 1998; von Eiff *et al*. 2001). Other methods useful for typing of staphylococci, as for example *spa* typing, MLST or multiple-locus variable tandem repeat (VNTR) analysis, would be also useful in typing of SCV *S. aureus* strains (Sabat *et al*. 1996; Enright *et al*. 2000).

Genotypic methods alone or in combination with classical immunological methods, declared for analysis of wild-type strains of *S. aureus*, can also detect structural genes or those encoding toxins (hemolysins, enterotoxins, superantigens, exfoliatins) in SCV *S. aureus* strains (Johnson *et al*. 1991; Lina *et al*. 1999; Becker *et al.* 2003; Fisher *et al.* 2007).

9 SUSCEPTIBILITY TO ANTIBIOTICS

Routine *in-vitro* susceptibility methods have been developed and approved for testing rapidly growing bacteria and because SCVs fail to meet the requirements the results should be interpreted with caution. Staphylococcal SCVs are more resistant to some classes of antibiotics than the parent strain. This is due to that an electrochemical gradient is required for the import of positively charged molecules, such as aminoglycosides (*e.g*., *S. aureus* SCVs are more resistant to gentamicin) and some lantibiotics, into the bacterium (Balwit *et al*. 1994). In addition, the slow growth of these organisms reduces the effectiveness of cell wall-active antibiotics, such a β-lactams (Devriese 1973; Schnitzer *et al.* 1943; Lacey and Mitchel 1969; Lacey 1969; Chambers and Miller 1987). Culture media containing various inhibitory factors, such as antibiotics, regularly yield SCV colonies which again revert in free media to the wild-type phenotype of *S. aureus* (Lacey 1969; Vesga *et al*. 1996; Wise and Spink 1953).

There is also a connection between antibiotic treatment and development of SCVs, because the formation and growth of SCVs are favored under the selection pressure. One of the early reported cases concerns a patient who suffered from chronic staphylococcal skin infections and was treated repeatedly for ten months with a combination of crystalline penicillin and oral chloramphenicol. The SCVs of *S. aureus* were cultivated from the pus of the patient by the end of the treatment period. Cultures were still positive even if the patient was free of antibiotics for more than a month (Goudie and Goudie 1955).

SCV positive patients usually belong into one of two groups, aminoglycoside resistant and sulfonamide resistant, according to the treatment. Gentamicin-resistant SCVs staphylococci are significant pathogens in patients with chronic staphylococcal bone infection, as the SCVs are selected by long-term therapy (von Eiff *et al*. 1997*a*). The bacteria are usually hemin and/or menadione auxotrophic. The subsequently reduced electron gradient across bacterial membrane decreases the uptake of aminoglycosides, thereby causing resistance (von Eiff 2008). These SCV strains have higher MIC to gentamicin or they are fully resistant to the drug even if single strains of SCV can be susceptible to the antibiotic (von Eiff 2008).

CF patients are highly susceptible to infection by SCV of *S. aureus* which were recovered from more than 25 % of the patients (Proctor *et al.* 1998). Because of preventive reasons or frequent exacerbations, patients are frequently and for long-term period treated by aminoglycosides and 'sulfa' drugs (Lyczak *et al.* 2002). SCV strains that usually evolve from the selective pressure in this case are thymidine (53 %) auxotrophs. These are resistant to 'sulfa' drugs (*e.g.* trimethoprim–sulfametoxazole) (Proctor *et al.* 1998). The mechanism of the resistance is caused by interference with the tetrahydrofolic acid pathway. The SCV strains can participate in etiopathogenesis of infectious processes if the host environment such as destroyed tissue or pus of CF patients, contains sufficient concentration of thymidine to saturate the bacterial requirements (Chatterjee *et al.* 2008). The method of choice to test auxotrophy to thymidine is broth-dilution method using brain heart infusion broth which contains high concentration of thymidine (Chatterjee *et al*. 2008).

MRSA with SCV phenotype has also been detected (Seifert *et al.* 1999). Combination of the resistance with phenotypic mimicry of the SCV variants is a real threat for infected patients. Susceptibility tests for detection of methicillin resistance could fail but results of genotypic analysis or latex agglutination of PBP2a confirm the resistance as in wild-type MRSA (Kipp *et al*. 2004).

10 INTRACELLULAR PERSISTENCE

An important part of the ability of SCVs to persist within the host probably relates to their ability to persist intracellularly in nonprofessional phagocytes, which shields them from the host immune response

(Proctor *et al.* 1995; von Eiff *et al.* 1997*b*, 2001; Garzoni and Kelley 2009; Sendi and Proctor 2009) (Fig. 8). Persistence is thought to be due to the reduced production of α-toxin (Balwit *et al*. 1994; von Eiff *et al.* 1997*b*). The importance of α-toxin production for cell lysis has been previously established with a site-directed mutants of the α-toxin gene or *hemB* gene – one of hemin biosynthetic genes (Menzies and Kernodle 1994; von Eiff *et al*. 1997*b*). The mutants, but not the parent strain, were able to persist within cultured host cells. While the mechanism for decreased production of α-toxin by *S. aureus* SCVs is unknown, most laboratory and clinical *S. aureus* SCVs produce nonhemolytic colonies, whereas almost all normally growing

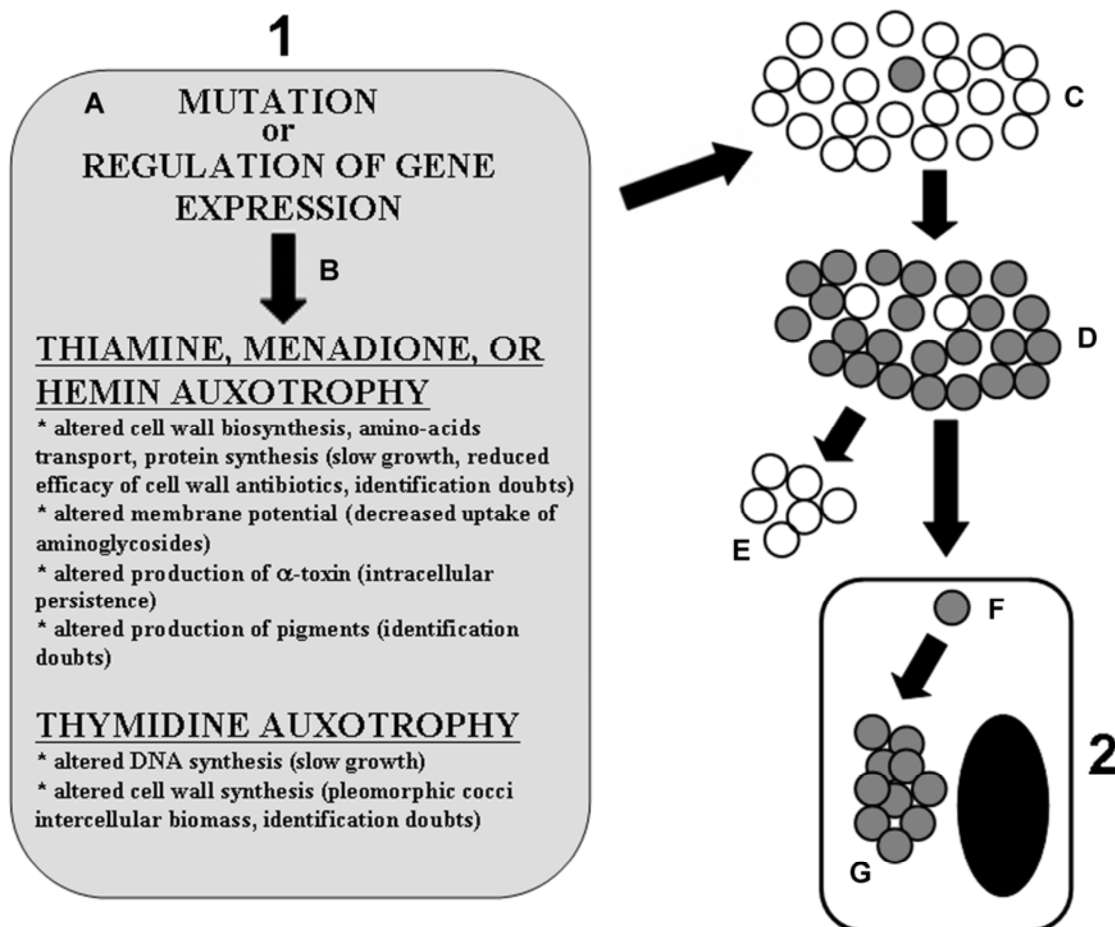

Fig. 8. Evolution of SCV *S. aureus* strain and intracellular persistence in host non-professional phagocytes. *S. aureus* cell (**1**) mutates in metabolic genes (**A**) causing auxotrophy to products (**B**) of the affected metabolic pathways. Expression of various genes could be regulated by non-protein coding RNAs (npcRNAs) which have integral role as ubiquitous regulators of gene expression. Target identification and functional studies are needed to determine the specific role of npcRNAs in formation of SCV *S. aureus* cells (*see also* Abu-Qatouseh *et al*. 2010). The mutated cell (*grey* cell in **C**) is progeny of SCV *S. aureus* subpopulation which outgrows parental population (**D**). Compared to extracellular life cycle of the parent population (**E**), SCV after internalization (**F**) and multiplying (**G**) can persist viable intracellularly in tissues consisting of non-professional phagocytes (*e.g*. endotelial cells) (**2**) (diagram – authors).

S. aureus clinical isolates are strongly hemolytic (Balwit *et al*. 1994; Browning and Adamson 1950; von Eiff *et al.* 1997*b*; Kahl *et al*. 1998; Quie 1969). Reduced α-toxin production seems to arise as a selective, rather than a generalized, response to decreased electron transport. The selectively reduced production results in two populations of organisms: an aggressive (α-toxin-producing) subpopulation that causes acute destruction of host tissues and a quiescent subpopulation that can persist within the protective environment of host cells yet cause recurrent disease when the electron transport chain is reconstituted. α-Toxin has been found as a key mediator of staphylococcal persistence in human monocyte-derived macrophages because it is essential for permeabilization of the phagolysosomal membrane (Sinha and Fraunholz 2010). Intracellular persistence of SCV staphylococci is also influenced by efficient invasion attributed to high expression of adhesin–fibronectin binding protein and down-regulation of α-toxin and proteinase which contribute to inflammation and tissue destruction (Tuchscherr *et al*. 2010). Except adhesins also platelets, plasma proteins, endo-

thelial cells and sub-endothelial tissue are involved in the *S. aureus* infection of vessel walls and the initial adhesion as well as the course of the infection are modulated by networks of different staphylococcal global regulatory systems, and activation of host cells and platelets (Sinha and Herrmann 2005).

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