congestion in the cities. Similarly, in zoos, university campuses and areas of tourist attraction like heritage sites, these rickshaws should be encouraged.

2. Since both MAPRA and IMPRA are environmentally sound and user-friendly vehicles, they should get all the financial benefits available to renewable energy projects. Besides, all the Government of India schemes for providing employment to weaker sections of society should be used to give loans to rickshaw pullers who want to drive these rickshaws.

3. There is a need for the concerned authorities in India to exempt MAPRA from the purview of Motor Vehicle Act since it is essentially a pedal rickshaw with a small motor.

Finally, it should be pointed out that the evolution of cities and towns has been driven by the transport system. The sprawling cities of the US developed because of automobiles. However, most European cities have integrated the public transport systems like rail, subway, bus and tram with private cars, taxis and cycles to cover the 'last mile'. This could also be possible in India where rickshaws can provide transportation to cover the last mile or kilometre. If we consciously promote vehicles which are human propelled, then we can help reduce the growth of cities and at the same time drastically reduce the energy used in transportation. This can show us a way towards a sustainable transport system of the future. I also hope that this article will generate interest in the large S&T community of the country to work for producing better cycle and electric rickshaws, which will help the environment and the lives of the poorest of poor of our country.

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REVIEW ARTICLES

Swarming: A coordinated bacterial activity

Manvi Sharma and S. K. Anand*

Dairy Microbiology Division, National Dairy Research Institute, Karnal 132 001, India

Swarming is an intrinsically surface-linked and cell density-dependent phenomenon involving cell differentiation, extensive flagellation, contact between neighbouring bacteria, and in particular, highly coordinated migration of swarm cells. Various extracellular compounds such as biosurfactants and polysaccharide also facilitate surface translocation. Recently, two regulatory systems, namely, Flagellar master FlhD-FlhC and AHLs-based quorum sensing systems have been identified, which play an important role in regulation of swarming behaviour. Interest in bacterial multicellular bahaviour is increasing not only as a focus for study of developmental regulation, but also in its role in virulence, biofilm formation and its connection with pathogenicity. So far, only a few of the *Serratia liquefaciens* quorum-sensing target genes have been identified. Some of these genes may encode potential virulence determinants, whose expression is sensitive to furanones. This may form a valuable model system for understanding the structure and function of bacterial signaling systems. The information on interaction of cognate signals with other modulatory signals may help to develop new strategies in the battle against infectious diseases.

UNTIL recently, bacteria were considered as unicellular organisms that grew and multiplied independently of each other. However, bacteria can undergo cell differentiation when they grow in colonies. Recently, various intercellular communication systems have been discovered, which indicate that bacteria are much more interactive than believed earlier and these communication capabilities are considered to be essential for coordinated

*For correspondence. (e-mail: skanand@indiatimes.com)

bacterial activity[1]. One such activity is the migration of bacterial population by a phenomenon referred to as 'swarming'[2].

Swarming is a special form of bacterial surface translocation dependent on the presence of surface but is different from other five modes of surface translocation including swimming, gliding, twitching, sliding and darting (Table 1)[3]. As early as 1885, Hauser described the phenomenon of swarming in the genus *Proteus*. When *Proteus* cells were inoculated at the surface of agar medium, regular colonies are formed at the inoculation point. Thereafter, cells at the rim of the colonies initiate a differentiation process resulting in long, multinucleated, aseptate, hyper-flagellated cells, which undergo cycles of rapid and coordinated population migration across the solid surface. Being analogous to swarming of bees, this type of multicellular bacterial behaviour is referred to as swarming motility[2]. The rapid outward movement of the swarm cells at the rim of swarm colonies is accompanied by bacterial growth inside the colony, resulting in fast colonization of all available surface.

The phenomenon of swarming is not limited to genus *Proteus*, but has also been demonstrated in a wide range of diverse bacteria (Table 2)[4]. All the swarming organisms possess peritrichous flagella, although vegetative cells may be either polar flagellated or having numerous lateral flagella[4,5]. Species capable of exhibiting this type of developmental behaviour lie on the boundary between unicellular and multicellular organisms[4].

The critical factors that determine whether the cells swim or differentiate into swarm cells or form regular colonies are the concentration of agar and viscosity (Table 2). On media containing low agar concentration (less than 0.4%), the organisms exhibit swimming motility, while on media solidified with 0.4–1.2% agar (optimum 0.7%), the organisms swarm on the top of agar surface. Further increase in agar concentration in the medium results in inhibition of migration of organisms and consequently normal-sized colonies are formed[6]. *Proteus*, *Vibrio* and some species of *Clostridium* are able to form swarming colonies on the surface of 2% agar (Table 2).

Table 1. Different types of surface translocations

Kind of surface transaction	Motive force	Type of movement
Swarming	Flagella	(a) Spreading produced on relatively dry agar surface (b) Cells move together
Swimming	Flagella	(a) Spreading produced on very moist agar surfaces (b) Cells move separately
Gliding	Generated by individual cell	(a) High activity (b) Cells move together
Twitching	Generated by individual cell	(a) Low activity (b) Cells move separately
Sliding	Generated by cell community	Centrifugal force in expanding colony
Darting	Generated by cell community	Tension force in cell aggregates

Source: Modified ref. 3.

Table 2. Bacterial species that exhibit swarming

Bacterium	Flagella arrangement (vegetative/swarm-cell)	Agar concentration permitting swarming (%)	Rate of surface translocation (μm/min)
Proteus mirabilis	Peritrichous	2.0	950
P. vulgaris	Peritrichous	2.0	nd
Vibrio parahaemolyticus	Polar/Peritrichous	2.0	125
V. alginolyticus	Polar/Peritrichous	2.0	nd
Serratia marcesens	Peritrichous	0.7–0.8	88
Chromobacterium sp.	Polar/Peritrichous	0.6–1.2	92
Clostridium tetani	Peritrichous	2.0	670
C. novyi	Peritrichous	2.0	nd
C. bifermentans	Peritrichous	1.0	300
C. sporogenes	Peritrichous	1.0	78
Bacillus alvei	Peritrichous	2.0	120
B. cereus	Peritrichous	2.0	108
B. subtilis	Peritrichous	1.8	80
B. megaterium	Peritrichous	1.1	560

Source: ref. 4.

Stages of swarming phenomenon

There are three well-defined stages of swarming. These include differentiation of vegetative cells into swarm cells, migration of swarm cell population and consolidation.

Differentiation of vegetative cells into swarm cells

When cells are inoculated at the surface of agar, regular colonies are formed initially. Later on, cells at the periphery or rim of the colonies undergo differentiation to form long, multinucleate, aseptate, hyper-flagellated swarm cells due to activation of flagella production and repression of cell septation[4,6].

Migration of swarm cell populations

This process involves the movement of highly flagellated, elongated swarm cells as groups or rafts on the surface of agar. Additionally, cell-to-cell contact is found to be essential for this type of migration. This is supported by the fact that isolated swarm cells are unable to move across the agar surface[6].

Consolidation

After a period of migration, swarm cells undergo consolidation, i.e. reversion to normally flagellated, short vegetative cells. These vegetative cells are capable of re-initiating further cycles of swarming differentiation and migration, giving rise to concentric zones on the surface of agar[2,4]. The various stages are depicted in Figure 1.

However, different organisms show different consolidation patterns, e.g. mutants of *Proteus mirabilis* and wild type of other bacterial species like *Clostridium sporogenes* and *Vibrio parahaemolyticus,* showed dentritic fractal consolidation pattern, while in the case of *Bacillus subtilis* and *Clostridium tetani,* no apparent consolidation zone was observed[4,6–8].

Not much is known about the exact mechanism of consolidation, but it has been found that a 50 kDa metalloprotease plays an important role in swarming probably by regulating re-initiation of septation and cell division during the process[4].

Influence of swarming behaviour in mixed consortia

It was found to be difficult to study the pattern of growth of different microflora in mixed species cultures. This is due to the fact that some of the bacteria like *Proteus,* that could show swarming behaviour, resulted in large spreads, covering the entire area of plate, thereby restricting the enumeration of other bacteria of the consortia.
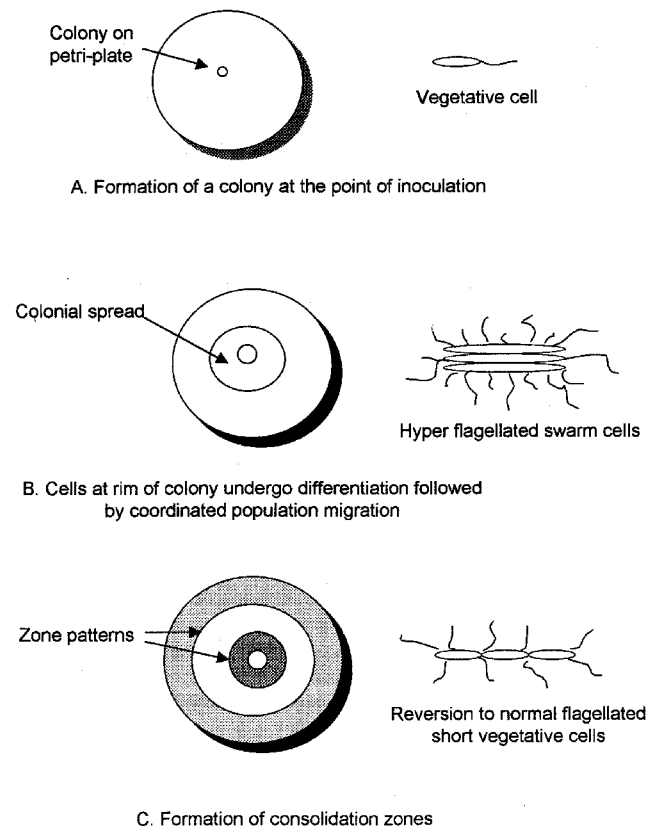
Several techniques were developed to restrict swarming while studying mixed species consortia.

Techniques that physically restrict movement of cells. The use of agar overlays has been recommended to inhibit swarming. However, this was found to be inapplicable in the isolation of obligate aerobes; moreover, typical colonial morphology of some organisms may be altered. Also, this technique does not always prevent swarming in *Proteus* around the edge and on the upper surface of the overlay[2].

Reduced surface moisture. As swarming is dependent upon the surface moisture, use of dried agar plates or agar plates treated with alcohol prior to inoculation have been recommended to prevent this phenomenon. However, this method is not very reliable as the dryness required to inhibit swarming, would completely suppress the growth of fastidious organisms[9].

Growth inhibitors. Several antibiotics such as neomycin, sulfonamides, combination of polymyxin and inhibitors such as chloral hydrate and sodium azide were also found to suppress swarming. The relative efficiency of several metabolic inhibitors had been determined in terms of growth-inhibiting concentration (GIC)/swarm

A. Formation of a colony at the point of inoculation

B. Cells at rim of colony undergo differentiation followed by coordinated population migration

C. Formation of consolidation zones

Figure 1. Stages of multi-cellular swarming behaviour.

cell-inhibiting concentration (SIC) ratio. GIC/SIC ratio was reported to be 15.0 for streptomycin and 27.0 for proflavin[2–5].

Inhibition of flagellar function. Several compounds were reported to inhibit swarming by disturbing the structural integrity or activity of flagella. For instance, addition of ferrous salt to proteose peptone medium inhibited the swarming of *Proteus* without affecting its growth, and *Proteus* cells grown in an equivalent liquid medium were nonmotile[10]. Similarly, boric acid inhibited the swarming on blood agar medium without affecting the growth of *Neisseria gonorrhoeae*. Boric acid was shown to form a complex with the flagellar protein that caused disintegration of flagella[2]. Sodium alkyl sulphate (a detergent) was also reported to be an effective anti-swarming agent and its efficiency increased with increasing chain length (GIC/SIC = 10). At the same time, ß-phenethyl alcohol (GIC/SIC = 5.0) and EDTA at a concentration of 2 mM or higher was also found to inhibit flagellation and thereby prevent swarming[2].

Inhibition by unique mechanism. Inhibition by some yet unexplained mechanisms has also been reported. In this category, the use of electrolyte deficient media for inhibition of swarming has been recommended. Activated charcoal at a concentration of 0.5% w/v or higher was also reported to inhibit swarming. Although PNPG (*p*-nitrophenyl glycerol) did not affect flagellation or motility, it could inhibit differentiation, thereby acting as an effective anti-swarm agent[11].

Factors responsible for swarming

Different studies have been carried out from time to time in order to determine the factors responsible for swarming. It was found that flagella, chemotaxis and slime play important roles in this type of behaviour[7,8].

Role of flagella

Swarming motility is driven by the movement of peritrichously arranged flagella, which are thought to function as helical propellers driven by a biological rotary motor[12].

In the case of *Serratia marcescens*, two cell forms exist that display two different kinds of motility; swimming and swarming, depending on the type of growth surface encountered. In liquid medium the bacteria are short rods with few flagella and show classical swimming behaviour. Upon growth on a solid surface (0.7–0.85% agar), they differentiate into elongated multi-nucleate flagellated forms that swarm over the agar surface. The flagella of swimmer and swarmers cells are composed of same 'flagellin' protein. The disruption of *hag* gene encoding

'flagellin', abolishes both swimming and swarming motility. All flagella negative mutants (Fla⁻), devoid of flagella and motility negative mutants (Mot⁻), having paralysed flagella, were defective in both swimming and swarming, indicating the importance of flagella. On the other hand, Che⁻ (chemotaxis defective) mutants having flagella show swimming, but no swarming. This suggests that an intact chemotaxis system is also essential for swarming in addition to flagella[8,12].

Role of chemotaxis

A functional chemotaxis system is essential for the swirling movement that is characteristic of swarming.

In order to explain the phenomenon of swarming, a hypothesis of negative chemotaxis was given by Lominski and Lendrum[13], also referred to as 'The theory of negative chemotaxis'. According to this theory, cells that are actively growing and dividing in the central colony produce and excrete a toxic metabolite that diffuses into the agar and establishes a gradient of decreasing concentration away from the colony. When the concentration of the metabolite reaches a critical level, it stimulates the formation of swarm cells by inhibiting cell division and stimulating flagella synthesis. The swarm cells once formed, are able to detect the toxic product and move away from the central colony and down the gradient in a negative chemotactic response. Upon reaching an area of plate where the level of metabolite is low enough, movement stops and the swarm cells divide to form normal short rods. These cells continue to grow and the cycle is repeated with a new generation of swarm cells emerging from the edge in first band and resulting in the formation of consolidation zones over the entire surface of the plate[2].

Evidence in support of theory of negative chemotaxis

Proteus has the unique ability to produce volatile amines (resulting from decarboxylation of amino acids) and specific growth-inhibiting metabolites that can induce swarming[14]. When *Proteus mirabilis* cells were removed from agar surface after 4 h of incubation (before the development of swarmers), washed and replated onto a fresh agar medium, they continue to differentiate into swarmers. This suggests that toxic metabolite responsible for induction of swarmers does not act as a non-specific inhibitor of division[15]. Similarly, activated charcoal adsorbs the toxic waste products and, therefore, prevents the swarm cell formation[14].

Limitations of negative chemotaxis theory

Certain facts however, still remain unexplained despite this theory. It was noticed that various treatments such as

penicillin (1–2 U/ml), H_2O_2, lithium chloride and UV irradiation could also induce the formation of elongated forms. However, these elongated cells were found quite distinct from swarm cells with respect to their length, shape, conformation of nuclear material and condition of cell wall[2]. Assuming 2.5 μm as normal length for broth grown cells, the peroxide treatment induced elongated cells upto 18 μm length, a value significantly less than maximum cell length of 80 μm reported for swarmers[16]. Similarly, motile mutants of *P. mirabilis* that had lost the ability to respond chemotactically (Che⁻ mutant) were still able to swarm[17]. On the other hand, PNPG (*p*-nitrophenyl glycerol) could completely prevent the swarming but had no effect on the chemotaxis of *P. mirabilis*. It was later explained that PNPG does not inhibit the movement of swarm cells (chemotaxis), but prevents their formation[15].

Role of slime

It has been reported that various species such as *Proteus mirabilis*, *Vibrio parahaemolyticus* and *Bacillus alvei* have the ability to produce extracellular slime which help in the migration of swarm cells over agar surface. *P. mirabilis* swarm cells produced a large quantity of extracellular slime (acidic polysaccharides) which formed a matrix between adjacent cells and encapsulated the cell aggregates or raft which was made up of the migrating cell mass. Therefore, it clearly indicates that slime plays an important role in the migration of swarm cells. This is supported by the fact that though non-swarming mutant of *P. mirabilis* fail to form slime, they produced flagellated long forms. The presence of various extracellular compounds such as biosurfactants and polysaccharides are found to facilitate the swarming[18,19].

Thus, extracellular slime played an important role in migration of bacteria upon solid surfaces referred to as the second stage of swarming.

Concepts in swarming

Recently, two key regulatory systems have been identified that are responsible for regulation of swarming behaviour[20]. These are: Developmental pathway, controlled by flagellar master FlhD-FlhC system and Biosynthetic pathway, controlled by AHLs (*N*-acyl L-homoserine lactones)-based quorum sensing system[11].

Developmental pathway: Flagellar master and cell differentiation

Flagellar master *flhDC* gene encodes the transcriptional regulators Flh D and Flh C, that control the expression of approximately 50 genes related to flagellar structure, chemotaxis and cell division[6].

A *flhDC* mutant of *S. liquefaciens* MG1, devoid of flagella, was unable to swim or swarm. Over expression of *flhDC* in liquid medium also induced the swarm cell differentiation, resulting in the formation of filamentous, multinucleated and hyper-flagellated cells that are indistinguishable from swarm cells isolated from the edge of a swarm colony. Thus, artificial stimulation of *flhDC* expression could overcome the obligatory requirement of surface contact. It clearly indicated that sensing of surface contact is a major stimulus for differentiation of vegetative cell into swarm cell (first stage of swarming) and is controlled by FlhDC operon. Recent studies with *P. mirabilis* and *S. liquefaciens* showed that FlhDC master operon was a major checkpoint for swarming behaviour in different bacteria[11].

All swarming bacteria possess peritrichous flagella, however the vegetative cells of *V. parahaemolyticus, V. alginolyticus* and *Chromobacterium* spp. possess polar flagella. The polar flagellated vegetative cells of *V. parahaemolyticus* are converted into peritrichous hyper-flagellated swarm cells by limitation of iron in the medium and contact with solid surfaces. The polar flagella of *V. parahaemolyticus* act as a tactile sensor, which detects forces that restricts its movement on surface and highly viscous media. This triggers the expression of swarm cell specific genes (*laf* genes) which form lateral swarm flagella[21,22].

Biosynthetic pathway: Quorum-sensing system

Microorganisms produce several chemical agents that are responsible for intercellular communication. When they reach a threshold, a specific response is induced. As response appears only when this specific level is reached, this phenomenon referred to as 'quorum sensing', i.e. the organism is able to sense when the population has reached the 'quorum'. The chemicals produced by microorganisms were identified as AHLs (*N*-acyl L-homoserine lactones)[1,23,24]. These were found to be the sole signalling agents involved in communication in various Gram-negative bacteria and responsible for various physiological responses such as bioluminescence in *Vibrio*, synthesis of exoenzymes in *Pseudomonas aeruginosa*, antibiotic production in *Erwinia carotovora* and pigment synthesis in *Chromobacterium violaceum* and swarming in various bacteria[1,25,26].

The phenomenon of swarming has been extensively studied in *Serratia liquefaciens*. This organism was reported to produce two extracellular signal molecules, namely, BHL (*N*-butanoyl L-homoserine lactone) and HHL (*N*-hexonyl L-homoserine lactone) that are responsible for sensing the density of population during swarming[19,20].

S. liquefaciens MG1 has two divergently arranged genes *swrI* and *swrR*. The *swrI* gene encodes AHL syn-

thase (a signal generator) that directs the synthesis of BHL and HHL in a molar ratio of 10:1. The *swrR*, on the other hand, transcribes convergently an open reading frame that codes for a polypeptide SwrR. The signal molecule BHL binds to SwrR, which in turn regulates the transcription of the *swrA* gene. The *swrA* encodes a peptide synthetase that catalyses the production of the surfactant serrawettin W2 (Figure 2). This surfactant is an extracellular lipopeptide (cyclic lipodepsipentapeptide carrying a 3-hydroxy C10 fatty acid side chain) which leads to reduction in surface tension[11]. Therefore, surface grown cells of *S. liquefaciens* create a conditioning film that changes the wettability and surface tension of the medium. The formation of this film is dependent on functional *swrI* and *swrA* genes. Genetic studies in *S. liquefaciens* showed that mutation of *swrI* gene leads to the inhibition of the swarming capability. However, swarming motility in a *swrI* mutant could be easily restored by supplementing the medium with 150 nM BHL or 900 nM HHL. BHL was found to be more efficient in promoting the swarming motility than HHL. The swarming motility of *swrI–swrA* double mutants of *S. liquefaciens* could also be restored by supplementation of the medium with 'serrawettin W2', a biosurfactant produced during swarming[6].

In *Pseudomonas aeruginosa*, a human opportunistic pathogen, the production of various extracellular virulence factors such as protease LasB elastase and rhamnolipid is controlled by las (*las I, las R*) and the rhl (*rhl I, rhl R*) quorum-sensing systems[27]. Recently, Kohler *et al.*[28] reported that swarming of *P. aeruginosa* requires rhamnolipid as a biosurfactant. Both *las I* and *rhl I* encode autoinducer synthases responsible for the synthesis of 3-oxo-C_{12}-homoserine lactone and C_4-homoserine lactone messenger molecules (autoinducers). These molecules accumulate at high cell density, activate their corresponding transcriptional regulator protein (LasR and RhlR, respectively) by forming a protein–inducer complex, leading thereby to a cell density-dependent activation of virulence gene expression[27,29]. Similarly, in *Serratia marcescens*, *N*-acyl homoserine lactones are synthesized due to expression of *smaI* (a *swrI* homologue)[30]. At the same time, *Bacillus subtilis* also produces a biosurfactant
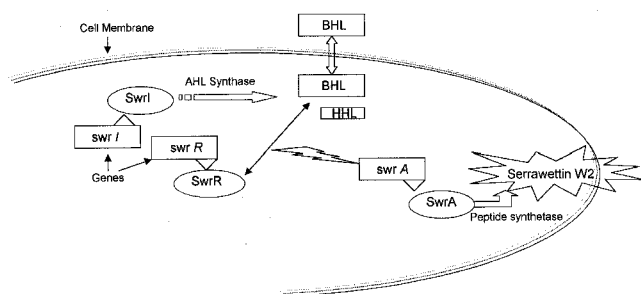

Figure 2. Quorum-sensing process mechanism in swarming.

'surfactin', a cyclic peptide consisting of 7-amino acids and 3-hydroxy 13-methyl tetradecanoic fatty acid side chain, responsible for swarming. *B. subtilis* mutants that are unable to produce surfactin have been demonstrated to be defective in swarming motility[31,32]. Therefore, it is clear that the presence of a biosurfactant is essential for coordinated population migration.

Hence, both developmental and biosynthetic pathways are collectively responsible for the swarming behaviour. Flagellar master *FlhD-FlhC* operon was found to be responsible for cell differentiation, i.e. development of hyper-flagellated, filamentous, multinucleated swarm cells from vegetative cells. When the swarm cells reach a high cell density (quorum), they stimulate the expression of the divergently arranged genes *swrI-swrR* and the promotor of *swrA* which encodes biosurfactant 'serrawetin W2'. This reduces surface tension and helps in the migration of swarm cells over the surface. The sequence of the events occurring in the two processes is summarized in Figure 3.

Cyclic dipeptides in Gram-negative organisms

Prasad[33] reported that bacteria also produced some signals other than AHLs that play a key role in modulating the same regulatory pathway induced by AHLs. A number of cyclic dipeptides have been reported, which could either stimulate or inhibit the swarming process. For example, cyclo (L-Pro-L-Tyr) produced by *P. aeruginosa* is a competitive inhibitor of BHL, and therefore, inhibits the swarming motility[34]. At the same time, cyclo (L-Pro-L-Met) produced by *Escherichia coli* stimulates the swarming motility of the *swrI* mutant as efficiently as BHL[6]. Another dipeptide cyclo (L-Phe-L-Pro) has been identified in *P. fluorescens* that acts on the central nervous system. Thus, cyclic dipeptides may influence interactions between bacterial pathogens and their hosts[33].

Unique characteristic of swarm cells

The swarm cells were found to be physiologically and biochemically distinct from vegetative cells. During differentiation of vegetative cells into swarm cells, there was increased production of a number of proteins such as flagella, urease, haemolysin and protease, that might be related to survival of the organisms. Many enzymatic activities such as tryptophanase, β-galactosidase, alkaline phosphatase are reduced during swarm cell differentiation and their levels again reach to normal level after consolidation (Table 3)[4]. Similarly, oxygen uptake, nucleic acid and protein synthesis are also reduced in swarm cells compared to vegetative cells[4].

In case of pigmented bacteria such as *Serratia* and *Chromobacterium*, the vegetative cells contain a bright orange pigment, whereas the swarm cells were found to
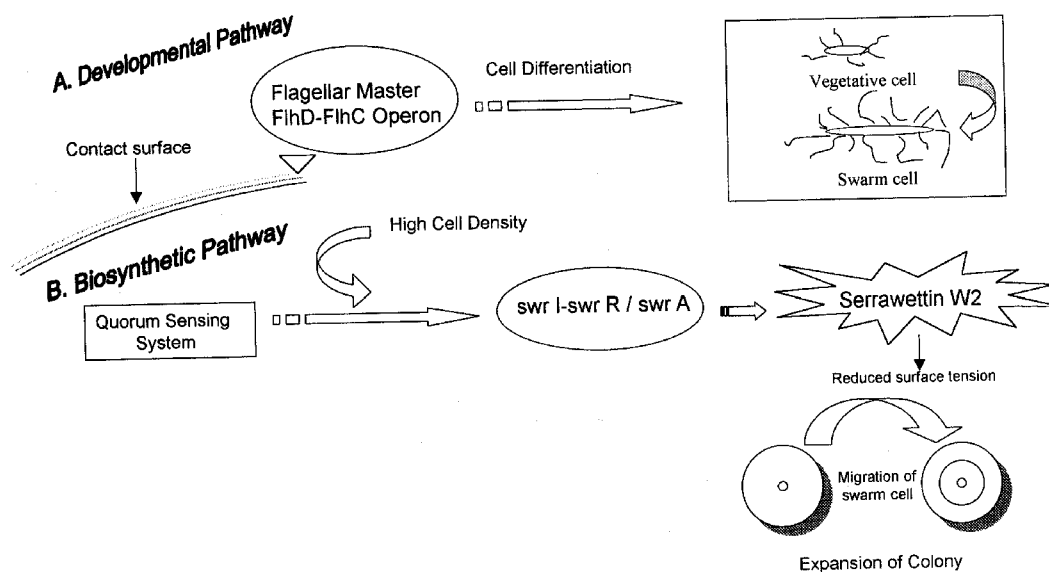
Figure 3. Schematic representation of pathways.

be completely colourless. However, they revert to the pigmented state after consolidation[4,5]. This process was reported to be due to the activity of a 50 kDa metalloprotease that plays an important role in re-initiation of septation[4].

Importance of swarming

Role of swarming in virulence

During swarming, long, multinucleated, hyper-flagellated and highly motile cells are produced that undergo rapid and coordinated population migration. These swarm cells have the ability to promote infection of host, e.g. *Proteus mirabilis* swarm cells could migrate through urinary tract and cause kidney infections[5,35], while the swarm cells of *V. parahaemolyticus* cause wound infections and septicemia[35]. *P. aeruginosa* caused cystic fibrosis and diffuse panbronchiolitis. The quorum-sensing autoinducer, 3-oxo-C_{12}-homoserine lactone produced by this organism was reported to have immunomodulatory activity and was responsible for damage to the lung tissues during these chronic infections[36,37]. Similarly, *S. marcescens* and *S. liquefaciens* cause respiratory and urinary tract infections due to swarming and are therefore considered as opportunistic pathogens[6].

Production of swarm-specific virulence proteins

A number of secreted proteins linked with virulence such as haemolysin, urease, and protease produced by swarm cells are responsible for various infections of susceptible hosts. Typical examples are the production of haemolysin

(Hpm A) produced by *P. mirabilis*, which damages mammalian cell membrane[4]. Similarly, urease generates NH_3, thereby elevating pH and results in the precipitation of salts to form kidney stones[35]. It has also been reported that increase in urease activity and haemolysin secretion of swarm cells results in 20-fold more cytolytic activity towards human uroepithelial cells cultured *in vitro*[4]. Metallo-protease has broad substrate specificity and could hydrolyse a variety of proteins including immunoglobulins (IgG, IgA) and basement membrane collagen[4,6].

Overproduction of these virulence factors during swarming has important consequences for the pathogenic potential of the organisms.

Ability to invade eukaryotic cell

Swarm cells not only produce virulence proteins, but also have the ability to invade mammalian cell lines or uroepithelial cells[4,6]. Even non-proteolytic or non-haemolytic mutants were shown to have the ability to invade eukaryotic cell lines. However, mutations affecting swarming motility significantly affected the bacterial invasion into human cells[4–6]. The differentiation of vegetative cells of *V. parahaemolyticus* into swarm cells plays an important role in its adsorption and colonization in chitinaceous shells of crustaceans[21].

Under conditions of co-culture growth, eukaryotes have been shown to develop chemical defence systems by producing secondary metabolites. For example, in case of *Delisea pulchra*, a marine algae which produces furanones (secondary metabolites) that act as signal inhibitors. These chemicals pass through bacterial membrane and compete with BHL for the binding site present

Table 3. Unique characteristic of swarm cell and vegetative cell isolated from different stages of swarming cycle

Characteristic	Cell type		
	Vegetative (V)	Swarm cell (S)	Consolidated (Vc)
Swarm cell-specific			
Flagella	++	+++	++
Haemolysin	++	+++	++
Urease	–	+++	–
Extracellular slime	++	+++	nd
Mammalian cell invasion	++	+++	++
Vegetative cell-specific			
Cell septation	++	+	++
Metalloprotease	++	–	+++
Oxygen uptake	++	+	++
Cytochrome synthesis	++	+	++
Alkaline phosphatase	++	+	++
β-Galactosidase	++	+	++
Chemotactic response	++	+	nd
Tryptophanase	++	+	++

+, Reduced level; ++, Normal level; +++, High level; –, Not detected; nd, not determined.
Source: ref. 4.

on *swrR*, thereby leading to inhibition of 'serrawettin W2' production and prevent the swarming in *Serratia liquefaciens*[38–40]. This process can be employed in controlling swarming behaviour.

Ability to form biofilms

In environmental, industrial and clinical situations most bacteria are to be found in organized biofilm communities colonizing surfaces, rather than growing in suspension as individuals[41,42]. The ability of pathogenic bacteria to form biofilm on transplants within the human body is a major medical problem[43]. In *P. aeruginosa*, flagella promote initial cell-to-surface contact, resulting in spread of growth, followed by formation of biofilm along abiotic surfaces[44]. Both 3-oxo-C_{12}-homoserine lactone and C_4-homoserine lactone autoinducers have been detected in significant amounts in pulmonary tissue obtained from a cystic fibrosis patient, as well as in the biofilm covering endotracheal intubation tubes obtained from patients colonized by *P. aeruginosa*[44]. Similarly, *P. mirabilis* can swarm over the surfaces and form crystalline biofims on urinary catheters[45,46]. The establishment of these biofilms results in blockage of the catheters and may lead to pyelonephritis, septicemia and shock. It has been reported that both biofilm formation and motility were related to the quorum-sensing system operating in the organism[41–47].

Conclusion

As swarming is a specialized form of surface translocation that involves differentiation of vegetative cells into

long, filamentous, multinucleated, aseptate, hyper-flagellated swarm cells that can coordinately migrate over solid surfaces, it provides a useful model system for the investigation of bacterial differentiation and multicellular behaviour. Moreover, bacterial virulence and development of biofilms have important consequences for metabolic and pathogenic potential of microorganisms. So far, only a few of the *S. liquefaciens* quorum-sensing target genes have been identified and as new details are available, it would be possible to have a full understanding of the biology and sociology of microbes.

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Gene expression during somatic embryogenesis – recent advances

Archana Chugh and Paramjit Khurana*

Centre for Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India

Somatic embryogenesis is a remarkable illustration of the dictum of plant totipotency. During embryogenic induction of cells, there is differential gene expression resulting in synthesis of new mRNAs and proteins. This genetic information in turn elicits diverse cellular and physiological responses that are involved in 'switching over' of the developmental programme of the somatic cells. Various model systems have been widely investigated to understand the mechanisms of gene regulation during this developmental process, and an array of genes activated or differentially expressed during so-matic embryogenesis have been isolated employing various molecular techniques. Nonetheless, the precise mechanisms controlling plant gene expression and the detailed steps by which these genes direct the plant-specific process of somatic embryogenesis remain far from being clearly understood. Thus, future trends involve characterization of development-specific genes during somatic embryogenesis to provide a deeper insight in understanding the mechanisms involved during differentiation of somatic cells and phenotypic expression of cellular totipotency in higher plants.

SOMATIC embryogenesis is the developmental restructuring of somatic cells towards the embryogenic pathway, and forms the basis of cellular totipotency in higher plants. This developmental switching involves differential gene expression conferring on the somatic cells the ability to manifest the embryogenic potential[1,2]. Somatic embryogenesis thus involves a plethora of molecular events encompassing not only differential gene expres-

*For correspondence. (e-mail: paramjitkhurana@hotmail.com)