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Synergistic interactions in the microbial world

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Abstract

After several decades of microbiological research has focused on pure cultures, synergistic effects between different types of microorganisms find increasing interest. Interspecies interactions between prokaryotic cells have been studied into depth mainly with respect to syntrophic cooperations involved in methanogenic degradation of electron-rich substrates such as fatty acids, alcohols, and aromatics. Partners involved in these processes have to run their metabolism at minimal energy increments, with only fractions of an ATP unit synthesized per substrate molecule metabolized, and their cooperation is intensified by close proximity of the partner cells. New examples of such syntrophic activities are anaerobic methane oxidation by presumably methanogenic and sulfate-reducing prokaryotes, and microbially mediated pyrite formation. Syntrophic relationships have also been discovered to be involved in the anaerobic metabolization of amino acids and sugars where energetical restrictions do not necessarily force the partner organisms into strict interdependencies. The most highly developed cooperative systems among prokaryotic cells appear to be the structurally organized phototrophic consortia of the *Chlorochromatium* and *Pelochromatium* type in which phototrophic and chemotrophic bacteria not only exchange metabolites but also interact at the level of growth coordination and tactic behaviour.

Types of cooperation among microorganisms

Microorganisms can cooperate in many different ways, and the mutual relationship of the partners to each other may vary from only marginal support to absolute mutual dependence. Many cases have been reported where one microorganism excretes metabolites, e.g., precursors of vitamins or certain amino acids which are used by a partner organism that lacks specific synthesis pathways and profits from this support, even if it could synthesize the respective compound on its own and this way only saves biosynthetic energy. More intense types of cooperation and mutual interdependence are found preferentially among anaerobic bacteria although we have to admit that we are biased in this view by the cultures we know: since bacteria are usually isolated with simple media that select for easy-to-cultivate organisms degrading a simple cocktail of substrate basically on their own we may overlook other bacteria that are outcompeted under such conditions and may display more refined types of interaction with others. Since we know only a small fraction of all microorganisms present in the environment, we cannot exclude that other bacteria out there might depend to a large extent on cooperations with partners, and perhaps just this is one of the reasons why we failed so far to cultivate them.

Contaminated and mixed cultures in the laboratory can provide interesting examples of interactions that establish in spite of all efforts of the experimentator to purify his cultures. Methanotrophic bacteria are often contaminated with methanol-oxidizing *Hyphomicrobium* sp. strains which profit from some excreted methanol and help protect the methanotroph by decreasing possibly toxic methanol concentrations (Wilkinson et al. 1974). Perhaps also formaldehyde is removed this way which is far more toxic than methanol. Another example are the well-known 'beards' of chemotrophic bacteria aggregating around heterocysts of cyanobacteria (Paerl & Pinckney 1996). No matter whether these partners live on excreted organic acids, amino acids, or molecular hydrogen, they consume oxygen and help this way to protect the heterocyst from intoxication by oxygen.

Whereas aerobic bacteria are usually considered to be able to degrade complex organic matter com-

pletely to CO_2 and H_2O this is true in the anaerobic world only in exceptional cases. Complex biomass is typically degraded in several steps, including classical (primary) fermentations, with subsequent further oxidation by sulfate reduction or iron reduction, or by coupling primary fermentations with secondary fermentations to methanogenesis at the very end (Bryant 1979; Schink 1997; Stams 1994; Zehnder et al. 1982). This kind of job-sharing among anaerobic microorganisms makes the whole process more complicated at first sight, but ascribes to every single organism only a limited task it has to fulfill.

Again, the interdependence among these partners may vary from an 'assembly line' type of cooperation called metabiosis in which only the later partner in the line profits from the former one but the advantage to the former members in the line by the later partners is negligible. Examples of this kind are degradation of glucose via acetate to methane by cooperation of *Acetobacterium woodii* and *Methanosarcina barkeri* (Winter & Wolfe 1979) and complete oxidation of trimethoxybenzoate via gallic acid and acetate by a triculture consisting *of A. woodii*, *Pelobacter acidigallici*, and *Desulfobacter postgatei* (Kreikenbohm & Pfennig 1985). Degradation of sugars and polysaccharides by clostridia is influenced positively by cooperation with hydrogen-consuming methanogens which shift the fermentation pattern to more acetate formation and, with this, to higher ATP yields (Schink 1997). Degradation of such compounds in sediments or in well-balanced sludge digestors may proceed nearly exclusively through acetate plus hydrogen, with very little production of reduced side products such as butyrate. Excessive production of these reduced side products is found only in pure culture. Fermentation of hexoses to acetate, CO_2 and H_2 only is exergonic but does not yield sufficient energy to synthesize 4 ATP by substrate level phosphorylation. Hydrogen removal to a low concentration makes this reaction further exergonic to allow a fermentation according to this pattern. Perhaps there is a considerable number of primary fermenting bacteria out there that can ferment sugars only to acetate, CO_2 and H_2, but they have been overlooked because our usual isolation strategies select for those organisms that can switch to a different fermentation pattern in pure culture. Finally, there are the strictly syntrophic relationships in which both partners depend on each other for energetic reasons and perform together a fermentation process that neither one of both could run on its own, as typical of syntrophic associations (Schink & Stams 2002).

Syntrophic associations in methanogenic cooperation

The peculiarities of obligately syntrophic cooperations in methanogenic degradation of primary alcohols, fatty acids, certain aromatic compounds, etc. have been discussed repeatedly with respect to their specific energetical problems and the biochemical solutions they found to solve them (Lovley et al. 1999; Schink 1997; Schink & Stams 2002; Stams 1994). In all these cases, the partner organisms have to share a very small energy budget, leaving only fractions of an ATP equivalent per reaction run for the partner organisms involved. In all cases studied so far, this can be accomplished by combinations of substrate level phosphorylation with reinvestment of ATP fractions, typically in reversed electron transport processes. The situation is most delicate with syntrophic associations degrading fatty acids such as butyrate, long-chain fatty acids, propionate, or acetate which leave the absolute minimum of 1/3–1/4 ATP equivalent (corresponding to 15–20 kJ per reaction run) to every partner. Syntrophic acetate conversion to methane and CO_2 can yield this minimum amount of energy even only at enhanced temperature: the reaction operates at its lower temperature limit at 37 °C (Schnürer et al. 1996) but runs far better at 55–60 °C (Hattori et al. 2000; Zinder & Koch 1984). The energetical situation of syntrophic ethanol conversion to methane plus CO_2 is considerably easier but so far we do not have a convincing concept how energy sharing between the partners is accomplished at the biochemical level.

An energetical problem similar to primary alcohols arises with primary amines. The first step, oxidative deamination to the corresponding aldehydes, is hard to couple to proton reduction, and would need reversed electron transport in this first step, similar to the situation with ethanol oxidation. However, this process has not been studied yet in defined cultures. The same reaction is also the most difficult step in anaerobic oxidation of several amino acids: conversion to the corresponding 2-oxo acid releases electrons at -115 mV which would require for proton reduction again an energy investment by reversed electron transport. The energetic situation of amino acid fermenting bacteria in syntrophic associations with methanogens has just been started to be tackled (Schink & Stams 2002). It is not surprising that amino acid fermenting bacteria develop a broad variety of fermentation patterns, depending on the specific chemistry of the respective amino acids involved. In some cases, the difficult ox-

idation processes can be coupled efficiently with, e.g., glycine reduction in the same organism as exemplified by the stickland reaction. However, this view is rather narrow as the versatile metabolism of *Eubacterium acidaminophilum* (Zindel et al. 1988) shows. This bacterium can either combine the oxidative and the reductive part of the Stickland fermentation simultaneously, or run either one of both separate, either with a hydrogen-consuming partner organism or with hydrogen as external electron donor. Again, this example demonstrates that our preferential look at substrate transformations by pure cultures gives only an insufficient picture of the complex situation prevailing in natural communities.

Types of metabolite transfer

In most syntrophic methanogenic associations, hydrogen plays a predominant role as electron carrier between oxidative and reductive metabolic processes. Its small size and easy diffusibility make it an excellent candidate for such interspecies electron transfer reactions. Nonetheless, in several cases also formate has been shown to act as electron carrier through a formate/CO_2 cycle. Theoretical considerations indicate that the formate system has certain advantages in an aqueous phase, whereas hydrogen might be better suited as carrier in densely packed microbial aggregates (Boone et al. 1989). Both carrier systems might also operate simultaneously in one degradative process, or the bacteria may switch between both electron transfer channels depending on the environmental conditions prevailing. Syntrophic oxidation of long chain fatty acids profits as well by efficient removal of the coproduct acetate through the activity of acetotrophic methanogens, and the same appears to be true for all fatty acid degrading systems examined so far. In the exceptional case of isovalerate degradation, acetate transfer is probably even more important than hydrogen transfer, and the case of methanogenic acetone degradation gives an example of interspecies transfer of acetate only (Platen & Schink 1987; Platen et al. 1994). One could as well think of interspecies methanol transfer because also this substrate is utilized by methanogens, but there is so far no convincing example of such a cooperation in methanogenic degradation.

A recently described syntrophically acetate-degrading culture consists of the iron(III)-reducing *Geobacter sulfurreducens* and the fumarate- or nitrate-reducing bacterium *Wolinella succinogenes* (Cord-Ruwisch et al. 1998). This artificially combined syntrophic coculture oxidizes acetate with nitrate as electron acceptor and does so at high rate, obviously independent of interspecies hydrogen transfer. We found recently that the interspecies electron transfer in this coculture is accomplished by cysteine which establishes a cysteine/cystine cycle for electron transfer between both partners. This electron transfer through an organosulfur compound reminds of interspecies electron transfer between a green phototroph and a chemotrophic sulfur-reducing bacterium in the association '*Chloropseudomonas ethylica*' (Biebl & Pfennig 1978) which cooperates through an H_2S/S_0 cycle.

Interspecies electron transfer has gained new interest through the discovery that microbial iron(III) reduction in natural environments can be mediated by humic compounds (Lovley et al. 1996); in the laboratory, usually anthraquinone-2,6-disulfonate is used as a model substrate. Several fermenting bacteria, e.g., *Propionibacterium* sp. can reduce such external electron carriers (Benz et al. 1998; Emde & Schink 1990) and can deliver electrons this way indirectly to Fe(III) minerals as well although they have never been regarded as iron-reducing bacteria. Electrons from quinoid carriers can as well be taken up by, e.g., nitrate-reducing bacteria or others (Lovley et al. 1999), and humic compounds can thus mediate electron transfer systems between rather different types of bacteria that would usually not be thought of as cooperation partners.

Phototrophic consortia

The highly organized syntrophic associations of phototrophic bacteria with colorless chemotrophic partners in the consortia '*Chlorochromatium*' and '*Pelochromatium*' (Pfennig 1980) represent exciting examples of a refined cooperation between metabolically different groups of anaerobic bacteria although the kind of cooperation between the partners is still enigmatic. Based on the discovery of interspecies electron transfer through a sulfur cycle between phototrophs and chemotrophs in the undefined coculture '*Chloropseudomonas ethylica*' (Biebl & Pfennig 1978), it was assumed that also in these consortia electron transfer proceeds through sulfur compounds, but this assumption could never be verified because no cultures of these consortia were available. Fröstl & Overmann (1998) have recently tackled this diffi-

cult issue again and obtained highly enriched cultures of '*Chlorochromatium aggregatum*' through enrichment based on skillful chemotaxis studies. It turned out that the consortium can be enriched with 2-oxoglutarate to which it is chemotactically attracted (Fröstl & Overmann 1998). 16S rRNA probing revealed that the chemotrophic central bacterium in these consortia belongs to the β-proteobacteria and is highly unlikely to reduce sulfur compounds (Fröstl & Overmann 2000). Thus, the concept of a sulfur cycle as basis for this cooperation has to be revised. One could think of an electron transfer system based on a 2-oxoglutarate/succinate cycle which involves a key enzyme of CO_2 fixation in the green phototroph, but such speculations need to be substantiated by experimental data. In any case, the successful cultivation of the first representatives of these exciting consortia is a key step for an understanding of these exciting syntrophic associations.

Anaerobic methane oxidation

Oxidation of methane coupled to sulfate reduction under strictly anoxic conditions has been postulated for many years to be an important process in the overall electron flux within anoxic marine sediments (Valentine & Reeburgh 2000). Nonetheless, all efforts to isolate bacteria catalyzing this exergonic reaction failed. Indications that this process is mediated through a syntrophic cooperation between a methanogen operating in reverse and a sulfate reducer (Hoehler et al. 1994) were based on observations that methanogens themselves can oxidize methane simultaneously with methane formation (Zehnder and Brock 1979). On the basis of the reaction energetics it was argued that the small energy gain available in this process ($\Delta G^{\circ\prime} = -18$ kJ per mol) could feed only one partner at maximum whereas the second one obviously had to run its activity cometabolically, thus preventing enrichment of both partners by conventional enrichment techniques (Platen et al. 1994). Indeed, the concept of a syntrophic cooperation between methanogens and sulfate reducers could be verified recently with the discovery of structured microbial aggregates in marine sediments covering methane gas hydrates (Boetius et al. 2000). These aggregates are very small, at maximum 10 μm in diameter, and exhibit a high degree of structural organization, with the methanogens in the center and the sulfate reducers at the periphery. They represent the first experimental basis for an understanding of anaerobic methane oxidation that can now be studied in the laboratory. Nonetheless, it has to be realized that these aggregates operate *in situ* under conditions with methane pressures up to 100 bar that are energetically far more favorable than the conditions for anaerobic methane oxidizers active in other marine sediments. Thus, future has to show if the consortia harvested at methane hydrates are representative for sulfate-dependent methane oxidation in general.

Microbial pyrite formation

In attempts to enrich for bacteria gaining energy for their metabolism from the conversion of ferrous sulfide with hydrogen sulfide to pyrite, according to

$$FeS + H_2S \longrightarrow FeS_2 + H_2 ,$$

we enriched a transferable microbial culture forming methane in stoichiometric amounts to the expected hydrogen formation. After several transfers, we found sulfate-reducing bacteria at comparably high numbers ($>10^8$ cells per ml) in these enrichment cultures, together with fluorescent methanogens. Inhibitor studies revealed that the above reaction was greatly enhanced by hydrogen removal through the methanogenic partner. So far, it remains unclear how the sulfate reducers can run an energy metabolism in these cultures, but since they obviously multiply to a significant extent they must have a metabolic advantage from their activity. Unfortunately, growth of these cultures is slow, and we did not yet succeed in isolating both partners and composing an active defined coculture.

Outlook

The few examples mentioned here should illustrate that there are many different types of cooperation between prokaryotes, and that in nature any kind of cooperative exchange of metabolites, etc., with partner organisms may be more the rule than the exception. I have concentrated in this survey mainly on symbiotic cooperations among anaerobic bacteria. Nonetheless, there are also many cooperations between prokaryotes and eukaryotes, not only in the anaerobic world, and there are further types of mutualistic cooperations between anaerobes and aerobes. An exciting example of this type is the close spatial association between sulfide-oxidizing and sulfate-reducing bacteria found

in marine microbial mats (Fukui et al. 1999). Cooperations of this type, where anaerobes transfer reduced degradation intermediates to aerobes, be they inorganic or organic, with the aerobe protecting the anaerobe from excess oxygen intoxication, may be widespread and have to be unraveled in the future. From this point of view, also the cooperation between higher animals and their anaerobic gut microbiota represents such a cooperative system in which the anaerobe helps to improve food utilization and transfers metabolites, e.g., fatty acids, to the animal host to fuel its energy metabolism, and the host protects the anaerobes from toxic oxygen. There are many, many more examples of this kind out in nature, and we only have to look at things in such broader terms to widen our eyes for the unexpected.

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