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Stress and the periodontal diseases: growth responses of periodontal bacteria to *Escherichia coli* stress-associated autoinducer and exogenous Fe

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Psychological stress is known to increase the circulating levels of the catecholamine hormones noradrenaline and adrenaline, which have been shown to influence the growth of a large number of bacterial species by acting in a siderophore-like manner or by inducing the production of novel autoinducers of growth. As we have previously demonstrated that periodontal organisms display differing growth responses to noradrenaline and adrenaline, the aim of this study was to determine whether these growth effects were based upon either siderophore-like or autoinducer mechanisms. Initial inocula of 43 microbial organisms normally found within the subgingival biofilm were established under anaerobic conditions (35°C). Each strain was re-inoculated into a serum-based minimal medium and growth was assessed by optical density (OD_{600nm}) with test and control cultures performed in triplicate. Test cultures were supplemented with either 50 μM ferric nitrate or a previously described *Escherichia coli* autoinducer of growth. Significant growth effects for supplementation with ferric nitrate (13 species responding positively) and *E. coli* autoinducer (24 species responding positively) were observed, with differences in growth response within bacterial species and within microbial complexes. When data for all organisms were compared with published responses to catecholamines there were only weak correlations with Fe ($r = 0.28$) and *E. coli* autoinducer ($r = 0.34$) responses. However, large positive responses (> 25% increase) to free Fe and/or *E. coli* autoinducer were significantly more prevalent in the group of organisms ($n = 12$) known to exhibit similar responses to catecholamine hormones ($P < 0.01$; $\chi^2 = 4.56$). The results support the view that catecholamines may exert their effects on subgingival organisms by initiating autoinducer production, or simply by acting in a siderophore-like manner, scavenging bound iron from the local environment. It is possible that autoinducer mechanisms may play an important role in the response of oral microorganisms to stress hormones, thereby contributing to the clinical course of stress-associated periodontal diseases.

Key words: autoinducer; catecholamine hormones; microbiology; periodontal disease; stress

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The primary aetiological agent in the initiation and progression of periodontal diseases is bacteria found within the plaque biofilm (24). In order to survive, each microorganism must have the ability to sense and respond to changes within its local environment (10) that may be invoked either by other microorganisms or by the host itself. Stress has been associated with many diseases and in particular a relationship between stress and infectious disease has been demonstrated (5).

The majority of studies have investigated the effects of stress on the immune system and postulated that a compromised host is increasingly likely to demonstrate disease in response to stress. Psychological stress has also been shown to increase the circulating levels of the catecholamine hormones noradrenaline (plasma level 300–600 pg/ml) and adrenaline (plasma level 30 pg/ml) (6) via hypothalamic corticotrophin-releasing hormone and subsequent adreno-corticotropic hormone production by the anterior pituitary gland. Research investigating the effects of noradrenaline and adrenaline within the gastro-intestinal tract has suggested that infective organisms may be able to utilise catecholamines to their advantage and in doing so cause significant damage to the host (2–4, 7). Further, these hormones have been shown to influence the growth of a large number of periodontal bacterial species (20). The mechanisms underlying these growth changes have yet to be fully explored. Data on organisms from the gastro-intestinal tract have indicated two possible mechanisms: the catecholamine hormones may induce the production of a novel series of autoinducers of microbial growth and/or virulence expression (2, 11) and/or act in a siderophore-like manner supplying sequestered iron (3). Bacterial autoinducers influence the growth characteristics of the organism producing them as well as other organisms found within the same environment. In this manner, noradrenaline and adrenaline have the potential to alter the microbial flora of the gastro-intestinal tract or periodontium and potentially increase patient morbidity.

Recent investigations have demonstrated that noradrenaline, at levels found within the gastro-intestinal tract postsurgery, can increase the growth of *Escherichia coli* in a serum-based medium through the production of an autoinducer of *E. coli* growth which does not belong to the well characterised families of autoinducers described in the literature (11). This novel heat stable autoinducer activity leads to marked increases in growth (usually 3 or 4 log increases) and virulence expression of many gram-negative organisms (2, 11). In a screen of 17 gram-negative and 6 gram-positive enteric isolates, the majority of gram-negative strains produced a heat stable autoinducer of growth in response to the catecholamine-induced *E. coli* autoinducer (2). In addition, gram-positive strains were also able to respond to stress-associated autoinducers (which showed a high degree of cross-species activity), but were unable to produce them (2). These data suggest the existence of a novel family of gram-negative bacterial signalling molecules that appear to differ from other well characterised autoinducers such as the homoserine-lactones (11), identified in naturally occurring biofilms (14), although it is currently uncertain whether *E. coli* autoinducer consists of single or multiple components.

Iron availability is essential to the survival of many bacteria as iron has a broad range of functions in microbial cellular metabolism (19). Organisms have become adept at obtaining iron from their hosts for growth (8, 25). In addition, iron also enhances bacterial virulence. Both of these functions are likely to play important roles in the pathogenesis of periodontal diseases (15). The concentration of iron within the human gingival crevice has been estimated in gingivitis and periodontitis to be 3.042 mg/l and 5.196 mg/l, respectively (17); however, under normal physiological conditions this iron will be bound within haemoglobin or other iron-binding proteins such as lactoferrin and transferrin. Noradrenaline has been shown to form stable complexes with both transferrin and lactoferrin, to antagonize their iron-withholding capacity and to facilitate the transport of iron into bacteria (3). Thus, hormones involved in the stress response may affect bacterial growth by liberating protein-bound iron.

Periodontal diseases are inflammatory lesions mediated by host parasite interactions, with in excess of 300 bacterial species implicated in their aetiology and pathogenesis (16, 23). Indeed, certain types of periodontal disease are thought to have stress as a 'risk factor' for their initiation and progression (18). Previously, our group has demonstrated that many bacterial species commonly found within the subgingival environment are able to respond to both noradrenaline and adrenaline (20). Therefore, it is possible that the positive growth effects of catecholamines on periodontal organisms detected *in vitro* might be due to their siderophore-like activity and/or their ability to induce the production of autoinducers of growth.

To investigate the possible mechanism(s) underlying the growth effects of catecholamines in subgingival organisms, this study examined bacterial responses to the noradrenaline-induced *E. coli* autoinducer and to exogenous iron. In this manner, we aimed to clarify whether an autoinducer mechanism cross-reactive with that demonstrated in the gastro-intestinal tract plays a role in the response of oral microorganisms to stress hormones (20) and/or whether the catecholamine-induced growth responses of potential periodontal pathogens may be due to the catecholamines facilitating iron transport into potential periodontal pathogens from sequestered host sources (3).

Materials and methods

Periodontal bacterial strains and culture techniques

A total of 43 microorganisms (ATTC strains) were investigated (Table 1) and cultured according to previously described methodology (26). Each organism was grouped into a microbial complex (*Actinomyces*, purple, yellow, green, orange or red complex as previously described (21)), and screened for iron or *E. coli* autoinducer responsiveness as previously reported (20). The initial bacterial inoculum was grown under anaerobic conditions (System One Anaerobic Chamber, Innovative Technology, Newburyport, MA; Atmosphere: 80% nitrogen, 10% carbon dioxide, 10% hydrogen) at 35°C. Exceptions to this protocol were *Porphyromonas gingivalis* and *Tannerella forsythia*, which were grown on TSA plates supplemented with 5 μg/ml hemin (Sigma Chemical Co., St. Louis, MO), 0.3 μg/ml menadione (Sigma) and 10 μg/ml N-acetylmuramic acid (Sigma). Initial inoculae for *P. gingivalis* and *T. forsythia* were established in mycoplasma broth (BBL, Baltimore Biological Laboratories, Cockeysville, MD) supplemented with 5 μg/ml hemin (Sigma), 1 mg/ml glucose (Sigma) and either 0.3 μg/ml menadione (Sigma) for *P. gingivalis* or 10 μg/ml N-acetylmuramic acid (Sigma) for *T. forsythia*. *Eikenella corrodens* was grown in TSB supplemented with 50 μg/ml potassium nitrate (Sigma). All bacterial inoculae were grown to turbidity and the optical density ($OD_{600\ nm}$) of cultures was adjusted to 0.5 with spent medium prior to use.

Production of *E. coli* autoinducer

E. coli E2348-69 strain (Department of Microbiology, University of Leicester,

Table 1. Growth responses (percentage change) to supplementation with Fe or *E. coli* autoinducer

ATCC strain	Species/complex	Hours of growth	% change	
			Fe	autoinducer
Actinomyces species				
12102	*Actinomyces israelii*	24	−10.1	−2.9
12104	*Actinomyces naeslundii I*#	24	46.0**	1.6
23860	*Actinomyces gerencseriae*#	24	13.3	21.7*
43146	*Actinomyces viscosus*	24	2.3	69.1***
Purple complex				
10790	*Veillonella parvula*	24	1.3	58.5**
17929	*Actinomyces odontolyticus I*#	48	30.1***	58.1**
Yellow complex				
10556	*Streptococcus sanguis*	48	9.6	14.4
10558	*Streptococcus gordonii*#	48	−15.3	−4.7
27335	*Streptococcus intermedius*#	24	−10.1**	106.9***
35037	*Streptococcus oralis*	48	−15.2**	50.0***
49456	*Streptococcus mitis*	24	−7.4**	−4.1*
Green complex				
23834	*Eikenella corrodens*#	48	54.2*	114.6**
29523	*Actinobacillus actinomycetemcomitans a*	24	−3.4	−1.1
33485	*Capnocytophaga. ochracea*	48	−17.5**	−19.8**
33612	*Capnocytophaga sputigena*	24	24.5***	7.3*
33624	*Capnocytophaga gingivalis*	48	5.6	−18.3**
43718	*Actinobacillus actinomycetemcomitans b*	24	−7.4**	48.8***
Orange complex				
10953	*Fusobacterium nucleatum* subsp. *polymorphum*	48	51.0***	47.3***
25586	*Fusobacterium nucleatum* subsp. *nucleatum*	24	−9.6	16.1**
25611	*Prevotella intermedia*	48	0.0	−8.9
27823	*Streptococcus constellatus*	24	−8.2*	102.9***
33099	*Eikenella nodatum*#	48	20.0	63.3**
33236	*Campylobacter gracilis*#	48	156.3***	14.1
33238	*Campylobacter rectus*	48	0.0	19.0**
33270	*Peptostreptococcus micros*	24	72.3***	58.5***
33563	*Prevotella nigrescens*	48	−8.4	0.0
33693	*Fusobacteria periodonticum*#	48	30.4	0.0
49256	*Fusobacterium nucleatum* subsp. *vincentii*#	24	21.6	37.8**
51146	*Campylobacter showae*	24	0.4	8.2**
Red complex				
33277	*Porphyromonas gingivalis*	48	−5.3*	−9.6***
43037	*Tannerella forsythia*	48*	−3.9	−9.8
Others				
8486	*Eikenella limosum*	48	8.1*	11.1
11827	*Propionibacterium acnes*	24	3.3	−23.1*
11828	*Propionibacterium acnes*	24	26.2***	−9.2*
14201	*Leptotrichia buccalis*	24	19.0***	108.3***
19696	*Neisseria mucosa*	24	−3.7	14.6***
25175	*Streptococcus mutans*	24	15.3***	22.1***
25845	*Prevotella melaninogenica*	48	−11.8	−4.4
27337	*Peptostreptococcus anaerobius*	24	28.3***	26.7***
27824	*Gemella morbillorum*	24	18.5***	70.2***
33271	*Eubacterium saburreum*#	48	−40.0**	108.0*
33397	*Streptococcus anginosus*	24	−37.9***	89.1***
35308	*Prevotella denticola*#	48	13.8	24.6**

Paired *t*-test on corrected OD values. Experiments performed in triplicate on three separate occasions; $n = 9$. ***P-value < 0.001. **P-value < 0.01. *P-value < 0.05.
Organisms known to show a 25% or greater increase in growth in response to supplementation with 50 μM catecholamine (20).

UK) (2) was grown in Luria broth (Sigma) under 5% carbon dioxide–air until turbidity. 10^5 serial dilutions of this turbid culture were made with SAPI to establish a small initial inoculum (typically 10–100 colony-forming units (CFU)/ml) and added to the experimental medium at 10 μl/ml. Serum-SAPI contained glucose (2.77 mM), ammonium nitrate (6.25 mM), potassium phosphate (1.84 mM), potassium chloride (3.35 mM), and magnesium sulphate (1.01 mM), adjusted to pH 7.5 and supplemented with 30% (v/v) bovine adult serum (Sigma). Serum-SAPI minimal medium 25 ml was inoculated with the diluted *E. coli* strain and supplemented with 50 μM noradrenaline (Sigma). This culture was grown under 5% carbon dioxide–air for 24 h at 37°C (Fig. 1) and supernatant containing autoinducer was obtained by filtration through a 0.2 μm pore size syringe unit filter (Corning Inc., Corning, NY). To produce noradrenaline-free autoinducer, 1.25 ml of the supernatant was added to fresh serum-SAPI minimal medium that was inoculated with a 10^5 dilution of the *E. coli*. After incubation under carbon dioxide–air for 24 h at 37°C, the final conditioned media (now referred to as 'autoinducer'), containing a maximum of 2.5 μM noradrenaline, was filter sterilised, aliquotted and stored at −20°C. Control media were treated in the same manner, but without noradrenaline supplementation.

Iron and autoinducer supplemented media

Iron (ferric nitrate: Sigma) was prepared shortly before use as a 10 mM stock solution in distilled-deionised water and filter sterilised. Test cultures involved supplementation of the serum-SAPI medium with 50 μM Fe or 5%v/v autoinducer, with the negative control being the nonsupplemented experimental medium. Further supplementations, essential for the growth of *E. corrodens, P. gingivalis* and *T. forsythia* were as indicated previously.

Assay procedure and growth evaluation

An inoculum of 500 μl of each periodontal ATTC strain (corrected to 0.5 OD_{600nm}) was added to 40 ml of serum-SAPI and 1 ml cultures ($n = 3$) added to 24-well multidishes (Nunclon™ Nunc, Rochester, NY) containing 50 μM Fe (positive control), 5%v/v autoinducer or no supplementation (negative control). Cultures were grown statically under anaerobic conditions at 35°C and bacterial growth (early log phase) was determined by OD measurement (600 nm: Ultrospec III, Pharmacia, LKB Biotechnology, Uppsala, Sweden) following mechanical agitation at 24 or 48 h growth (see Table 1). Each OD reading was compared to a paired noninoculated control culture containing 50 μM Fe or 5%v/v autoinducer.

All cultures were performed in triplicate on three separate occasions. The optical densities of the noninoculated control cultures were subtracted from those of inoculated cultures to produce an OD

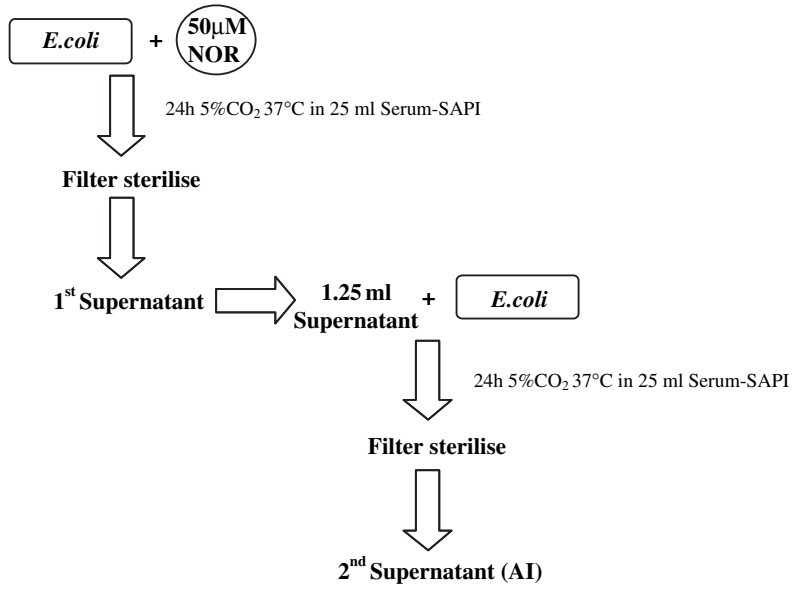

Fig. 1. Diagram illustrating *E. coli* autoinducer production.

reading minus any OD change arising due to the addition of each supplement. Non-inoculated controls also served as negative growth controls, ensuring that results were not due to contamination of media or supplements.

Data analysis

The growth response for all cultures was determined following subtraction of the noninoculated controls, and mean responses (OD_{600nm} change) for each organism were calculated. Finally, the OD_{600nm} change was converted to CFU/ml using the experimentally predetermined conversion factor for each organism which related the OD_{600nm} to CFU. Corrected OD_{600nm} readings or calculated CFU/ml for each supplemented culture were compared with non-supplemented controls using paired *t*-tests; *P*-values of ≤ 0.05 were accepted as significant. The relationships between responses to autoinducer, iron and catecholamines were investigated using Spearman rank correlation. The catecholamine responses used in these correlations were the raw data (mean OD_{600nm} change) from previously published experiments (20).

Results

E. coli responses

Large, significant ($P < 0.001$) increases in growth were observed in cultures supplemented with 50 μM Fe, 5%v/v *E. coli* autoinducer and 50 μM noradrenaline compared to nonsupplemented controls (Fig. 2).

Responses of the ATCC strains of periodontal bacteria to autoinducer and Fe

The responses of the 43 organisms (grouped as microbial complexes) investigated are shown in Table 1, which contains the corrected, percentage changes in growth relative to the unsupplemented control culture. Growth stimulatory and inhibitory effects were evident within and between microbial complexes with the magnitude of the growth response to autoinducer rarely reflected in the Fe responses. Reviewing the data for autoinducer responsiveness there were varied effects within and between each microbial complex, with organisms demonstrating mixed responses both within and across species. Two Actinomyces species responded positively from a total of four organisms within the complex. Positive responses were also recorded in both organisms from the Purple complex and in eight of the 12 Orange complex organisms. Negative growth effects for the Red complex organisms were recorded (one negative from two), demonstrating the degree of variation.

Overall there was no significant correlation between responses to autoinducer and Fe ($r = 0.28$). When data for all organisms were compared with published responses to catecholamines (20) there were low correlations between the catecholamines and Fe ($r = 0.28$) and *E. coli* autoinducer ($r = 0.34$), respectively, with the latter being significant ($P < 0.05$).

Responses of the Streptococci to autoinducer revealed two distinct groups (Fig. 3). One group (*Streptococcus sanguis*, *Streptococcus gordonii*, *Streptococcus mitis*) did not respond positively, whereas the other (*Streptococcus intermedius*, *Streptococcus oralis*, *Streptococcus constellatus*, *Streptococcus mutans*, *Streptococcus anginosus*) showed small to moderate significant increases in growth. In this group, except for *S. mutans*, Fe had a negative growth effect. Interestingly, *S. mutans* demonstrated the strongest growth within the unsupplemented experimental serum-SAPI medium.

All three subspecies of *Fusobacterium nucleatum*, a species known to have a pivotal role in the build up and biological properties of subgingival plaque (13), demonstrated small but significant positive responses to autoinducer (Fig. 4). By contrast, Fe only induced enhanced growth in *F. nucleatum* subsp. *polymorphum.*

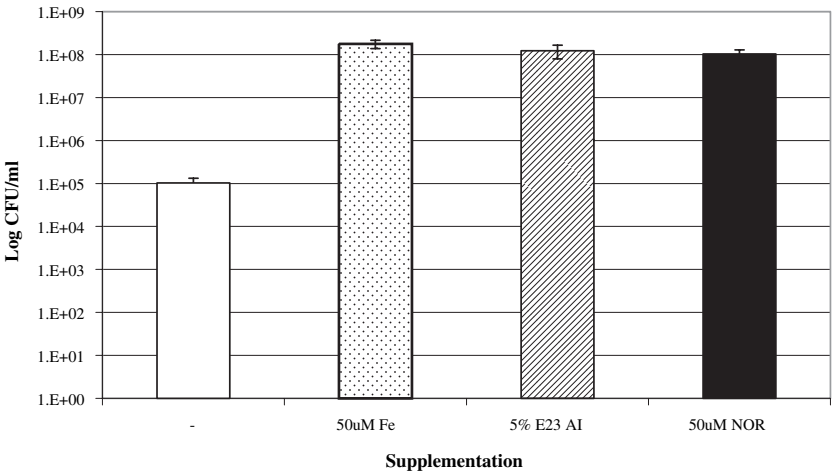

Fig. 2. Growth response of *E. coli* (E2348-6) at 24 h grown in 30% serum-SAPI medium supplemented with 50 μM Fe, 5%v/v autoinducer or 50 μM NOR (mean log CFU/ml ± SEM; $n = 9$).

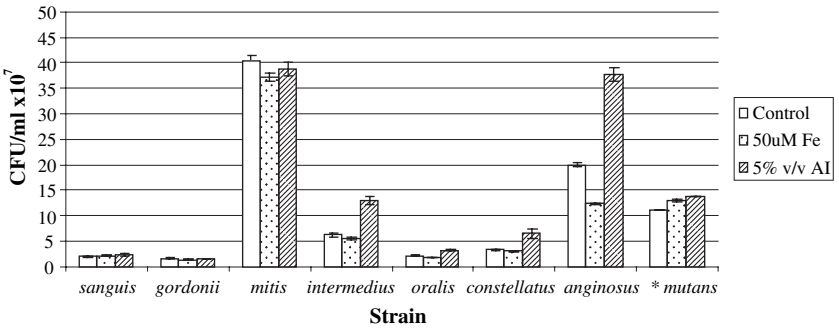

Fig. 3. Growth responses of streptococci found within the 'Yellow complex' grown anaerobically (24 or 48 h – see Table 1 for individual organism) in 30% serum-SAPI medium supplemented with 50 μM Fe or 5%v/v autoinducer (mean CFU/ml × 10^7 ± SEM; $n = 9$). *****Values for *S. mutans* are CFU/ml × 10^8.

Fifteen of the 43 strains tested gave >45% positive growth responses to *E. corrodens* autoinducer (Table 1) and of these, only three gave similar responses to supplementation with Fe (*E. coli*, *F. nucleatum* subsp. *polymorphum*, *P. micros*). Positive growth responses to Fe were generally less than those seen for autoinducer, with only five of the 43 organisms showing a >45% effect (*Actinomyces naeslundii I*, *E. corrodens*, *F. nucleatum* subsp. *polymorphum*, *Campylobacter gracilis*, *P. micros*).

Investigating the responses of *S. constellatus* and *F. nucleatum* subsp. *polymorphum* (Fig. 5a,b) in detail revealed that the inhibition of *S. constellatus* and the enhancement of growth of *F. nucleatum* subsp. *polymorphum* by Fe was dose dependent. By contrast, the enhancement of growth in both species observed with *E. coli* autoinducer reached a maximum at 0.3% supplementation. No further enhancement of growth was observed with increasing supplementations above 0.3%. The addition of equivalent volumes of nonsupplemented 'fresh' serum-SAPI had no effect on the growth responses observed, demonstrating responses identical to the nonsupplemented controls.

Discussion

This study has demonstrated, for the first time, that subgingival organisms are able to respond to a novel stress-associated autoinducer of growth produced by *E. coli*. The data support previous work performed on organisms found within the gastro-intestinal tract (2) as both gram-positive and gram-negative organisms were able to respond to the *E. coli* autoinducer. Thus the current data suggest that stress-related autoinducers similar to that produced by *E. coli* may play a role in the growth and development of the subgingival biofilm and be implicated in the pathogenesis of those periodontal diseases that are associated with human stress responses (9).

A major difference in the *E. coli* autoinducer-induced enhanced growth of subgingival plaque organisms and those derived from the gastro-intestinal tract is the magnitude of the response. Gastro-intestinal organisms under aerobic conditions invariably show substantial positive growth effects (2–4 log increases in 24 h), whereas those elicited in subgingival organisms are smaller and typically less than a doubling of growth in 24–48 h of anaerobic culture. The finding of only small increases in growth yield could

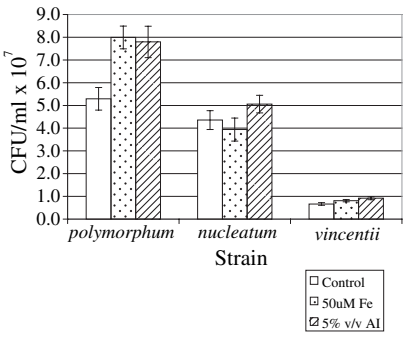

Fig. 4. Growth responses of the three subspecies of *Fusobacterium nucleatum* found within the 'Orange complex' grown anaerobically (24 or 48 h –see Table 1 for individual organism) in 30% serum-SAPI medium supplemented with 50 μM Fe or 5%v/v autoinducer (mean CFU/ml × 10^7 ± SEM; $n = 9$).

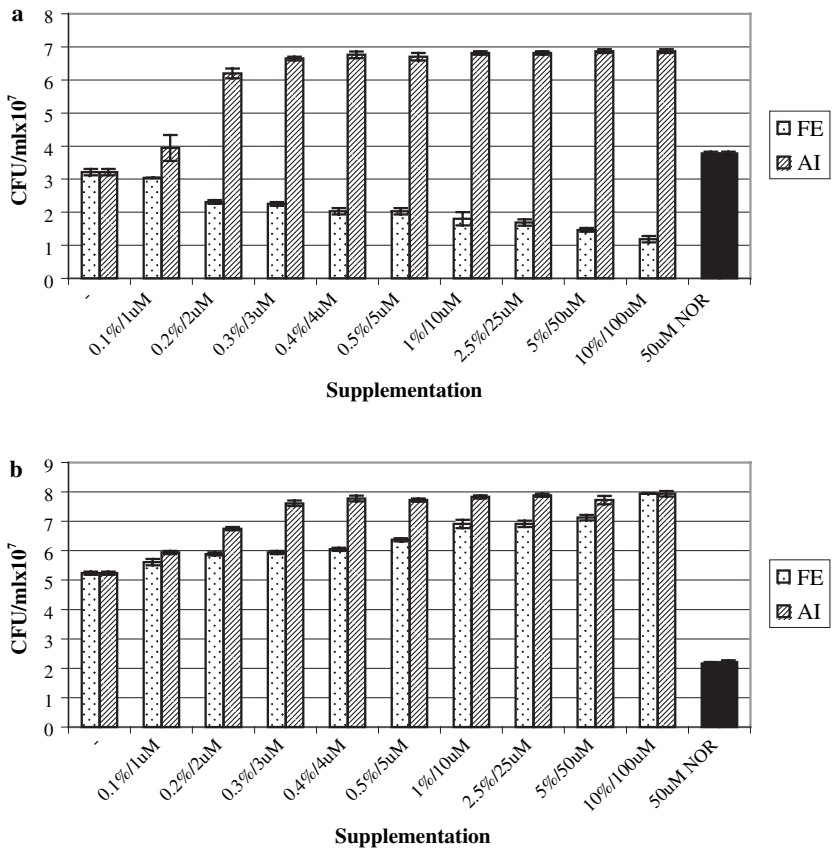

Fig. 5. Growth responses of (a) *Streptococcus constellatus* (24 h) and (b) *Fusobacterium nucleatum* subsp. *polymorphum* (48 h) to increasing amounts of Fe or autoinducer grown anaerobically in 30% serum-SAPI medium (mean CFU/ml × 10^7 ± SEM; $n = 9$). Black bars indicate responses to 50 μM noradrenaline.

indicate that the growth effects detected are due to the presence of a limiting nutrient within the spent medium rather than a true autoinducer response. That this is not the case was shown by dose–response experiments, using different amounts of autoinducer-containing spent medium on two of the responsive organisms (*S. constellatus* and *F. nucleatum* subsp. *polymorphum*), which revealed a typical autoinducer response that reached a plateau at 0.3% supplementation.

Although *E. coli* autoinducer does cause statistically significant increases in the growth of subgingival organisms at 24–48 h of exposure, it could be argued that such responses would not be biologically significant in respect of the pathogenesis of periodontal disease. However, further investigation (data not shown) has demonstrated that for subgingival organisms grown in serum-SAPI medium, 24–48 h incubation represents a very early stage in the growth curve and preliminary studies indicate that over longer time courses (96–120 h) more prolonged responses of a greater magnitude are seen. Further studies are planned to investigate the longer term effects of *E. coli* autoinducer, iron, noradrenaline and adrenaline upon periodontal bacteria strains to ensure that the magnitude of the responses reported here and previously (20) are not underestimating the true longer term effects, and to elucidate the effects of catecholamines and autoinducer on virulence factor expression within periodontal pathogens.

Our previous investigations involving the same 43 subgingival organisms studied here, have demonstrated that the catecholamine hormones have significant effects upon growth *in vitro* (20), with 12 species exhibiting similar levels of growth enhancement as found with *E. coli* autoinducer (species showing greater than 25% catecholamine-induced growth enhancement are identified in Table 1). The mechanisms underlying these stress-hormone effects are unknown, and it has yet to be determined whether the levels of catecholamine hormones can approach those found within the gastro-intestinal tract (50 μM) within inflamed (plaque-induced) periodontal tissues. However, research on gastro-intestinal tract organisms has suggested that there are at least two possible mechanisms underlying the growth promoting effects of catecholamine hormones: they may induce the production of a novel series of autoinducers of microbial growth and/or virulence expression (2, 11) or they may act by facilitating the uptake of iron from iron-binding proteins into bacterial cells (3).

The present studies were performed to investigate whether these mechanisms might explain the reported catecholamine-induced enhancement of growth in some subgingival organisms by examining relationships between growth responses to catecholamines, iron and *E. coli* autoinducer. The data for all 43 organisms show only weak correlations between catecholamine responses and *E. coli* autoinducer ($r = 0.344$; $P < 0.05$) and iron ($r = 0.283$; $P =$ NS). However, the responses within individual organisms allow the potential identification of species responding to catecholamines via autoinducer and/or siderophore-like mechanisms as well as organisms that may respond to *E. coli* autoinducer but are incapable of its production (Table 2). It is interesting to note that of the 12 species exhibiting greater than 25% growth enhancement in response to catecholamines, only three organisms failed to show similar growth enhancement in response to iron and/or *E. coli* autoinducer (*Actinomyces gerencseriae*, *Fusobacterium periodonticum*, *Prevotella denticola*). This was significantly different from the nonresponse rate in organisms that did not exhibit a greater than 25% growth enhancement in response to catecholamines (19 out of 31; $P < 0.01$; $\chi^2 = 4.56$).

Thus the data identify species for further study in evaluating the potential role of catecholamines and stress-related autoinducers of growth in the evolution of subgingival plaque and its pathological consequences. For example, both *S. intermedius* and *S. constellatus* demonstrate large responses to *E. coli* autoinducer and catecholamines. *S. intermedius* has been found to be elevated in microbial profiles previously reported for refractory periodontitis (7, 22), with *S. intermedius* and *S. constellatus* being of considerable importance in identifying patients with refractory disease (1). As stress has anecdotally been implicated as a contributory factor in the pathogenesis of refractory periodontitis, these growth changes, taken as a whole for catecholamines and *E. coli* autoinducer, may provide a potential mechanism underlying the clinical observations. It would be interesting to investigate the long-term effects of noradrenaline and adrenaline upon the dynamics of polymicrobial cultures of oral organisms and to investigate the abilities of putative periodontal pathogens to produce and respond to autoinducers found within the subgingival plaque biofilm.

In conclusion, this study has demonstrated for the first time that microorganisms found within subgingival plaque are able to respond to a novel, heat stable

Table 2. Classification of the 12 most catecholamine-responsive subgingival species based on the possible mechanism underlying growth enhancement (in conjunction with (20))

25% positive growth response to:			
Free Fe	*E. coli* autoinducer	Species showing a 25% increase in growth due to 50 μM adrenaline &/or noradrenaline	Possible mechanism (s) of action
+	+	*A. odontolyticus* *E. corrodens*	*E. coli* AI responder; catecholamine response due to siderophore-like activity &/or production of autoinducer
–	+	*S. intermedius* *E. nodatum* *F. nucleatum* subsp. *vincentii* *E. saburreum* *P. denticola*	*E. coli* AI responder; catecholamine response due to production of autoinducer
+	–	*A. naeslundii I* *C. gracilis* *F. periodonticum*	Catecholamine response due to siderophore-like activity &/or production of non-*E. coli* cross-reacting autoinducer
–	–	*A. gerencseriae* *S. gordonii*	Catecholamine response not due to siderophore-like activity but production of non-*E. coli* cross-reacting autoinducer

autoinducer of growth produced by *E. coli* in response to noradrenaline (12). This raises the possibility that autoinducer mechanisms may play an important role in the response of oral microorganisms to stress hormones, thereby contributing to the clinical course of stress-associated periodontal diseases. Recent estimates suggest that approximately 300 bacterial species are found within the subgingival environment (16) and the presence of an autoinducer of growth within this environment would have potential to increase the growth of certain organism(s) ahead of those that do not respond to such autoinducers. Further studies are underway to investigate the specific mechanisms of putative periodontal pathogens to the stress-related catecholamine hormones and to determine whether these responses are due to siderophore or autoinducer production.

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